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NAME OF AUTHOR/*NOM DE L'AUTEUR* MARTIN G. LAPP

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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. W. P. SKOROPAD

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APPRESSORIATION IN *COLLETOTRICHUM GRAMINICOLA*

by

© MARTIN S. LAPP

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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IN

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EDMONTON, ALBERTA

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled .....
APPRESSORIATION IN COLLETOTRICHUM GRAMINICOLA
.....
.....
submitted by Martin S. Lapp .....
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
in Plant Pathology

W. P. Skoropad
.....
Supervisor

.....

David D. Biss
.....

.....

.....

H. E. Green
.....
External Examiner

Date December 1, 1976.

ABSTRACT

Four aspects of the preinfection phase of Colletotrichum graminicola (Ces.) Wils. are reported in this study.

A mathematical model, which is a modification of the Gompertz equation, can be used to determine the number of germinations and of appressorial formations in a population of conidia of C. graminicola. It will determine both the maximum proportion of conidia which will germinate or which will form appressoria; and the time for these events to occur. The equation parameters which are functions of biotic and abiotic conditions are determined using a nonlinear regression programme. The data used to test the model show that continuous light on the parent fungal colony reduces subsequent conidial germination, but does not change the time taken to reach this maximum.

The location of appressoriation in C. graminicola was studied using natural and artificial (nail polish and epoxy) leaf surfaces. The distribution of appressoria relative to the groove over the anticlinal walls of the epidermal cells was the same for natural and artificial surfaces. This evidence shows that the location of appressoriation is controlled by the host topography and not by localized host exudations.

Regulation of appressoriation in C. graminicola was investigated using exogenous adenosine 3':5'-cyclic

monophosphate (cAMP), adenosine 5'-monophosphate (AMP), 1,3-dimethylxanthine (theophylline) and sodium fluoride (NaF). Each of these chemicals inhibited appressoriation but only NaF inhibited germination. Increased levels of cAMP have no effect on germination of the conidia but inhibit appressoriation.

The nature of the adhesive "mucilage" of the appressoria of C. graminicola was studied by testing the ability of NH_4 oxalate, NaOH or hemicellulase to release appressoria from glass cover slips on which they had formed. Because only NaOH or hemicellulase released appressoria, it is concluded that the adhesive factor is a hemicellulose.

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GENERAL INTRODUCTION

The fungus, Colletotrichum graminicola (Ces.) Wils., is the causal agent of anthracnose of many grasses. Its host range includes the common grain crops such as corn (Wheeler et al. 1974), oats (Sanford 1935), barley, wheat (Skoropad 1967), rye, Sudan grass (Bruehl and Dickson 1950) as well as many forage grasses. It can infect the roots, stem, leaves and seeds (Sanford 1935, Bruehl and Dickson 1950, Wheeler et al. 1974, Warren and Nickolson 1975). The perfect stage of C. graminicola which has been produced in artificial culture but has not been found in nature, has been identified as Glomerella graminicola (Politis 1975).

The life cycle of C. graminicola has been elaborated by Sanford (1935) and by Bruehl and Dickson (1950). Conidia constitute the main inoculum which is dispersed mostly by rain. The conidia germinate in a film of water to form germ tubes. An appressorium is formed almost immediately after the germ tube emerges or after some elongation has occurred. After the appressorium is mature, a penetration peg grows out of a pore on the bottom of the appressorium. This penetration peg grows through the host cuticle and epidermal cell wall, and once inside the epidermal cell the hyphae ramify throughout the host tissues. Later, as the host matures the mycelium forms setose acervuli which produce large masses of conidia. The conidia are released in an ooze under wet conditions and the cycle starts over again.

C. graminicola is thought to overwinter as mycelium in plant debris.

In this study only the prepenetration phase (FIG. 1) consisting of germination, germ tube elongation, and appressoriation (appressorial formation) was investigated.

Germination, appressoriation and penetration have been studied in C. graminicola with the light microscope (Skoropad 1967) and with the electron microscope (Politis and Wheeler 1973, Politis 1976). The conidia are uninucleate, hyaline single cells. With the addition of water and just prior to germination, the conidia swell, undergo mitosis and become two celled. A germ tube is formed from one or both cells. The appressoria are dark, club shaped, thick walled structures which are firmly attached to the leaf surface (Sutton 1968). They have characteristic shapes and sizes for different Colletotrichum spp. and are useful taxonomically (Sutton 1966,1968). It is uninucleate with a large nucleolus, many lipid bodies, mitochondria, ribosomes and a few small vacuoles. The penetration peg grows from the single pore on the bottom of the appressorium and uses both mechanical forces and enzymatic processes to make its way through the host cuticle and epidermal cell wall (Politis 1976).

Appressoria have recently been reviewed by Emmett and Parbery (1975). They define an appressorium as any structure "whose basic function is entry into a host". Skoropad (1967) found that 25C was the optimum temperature for

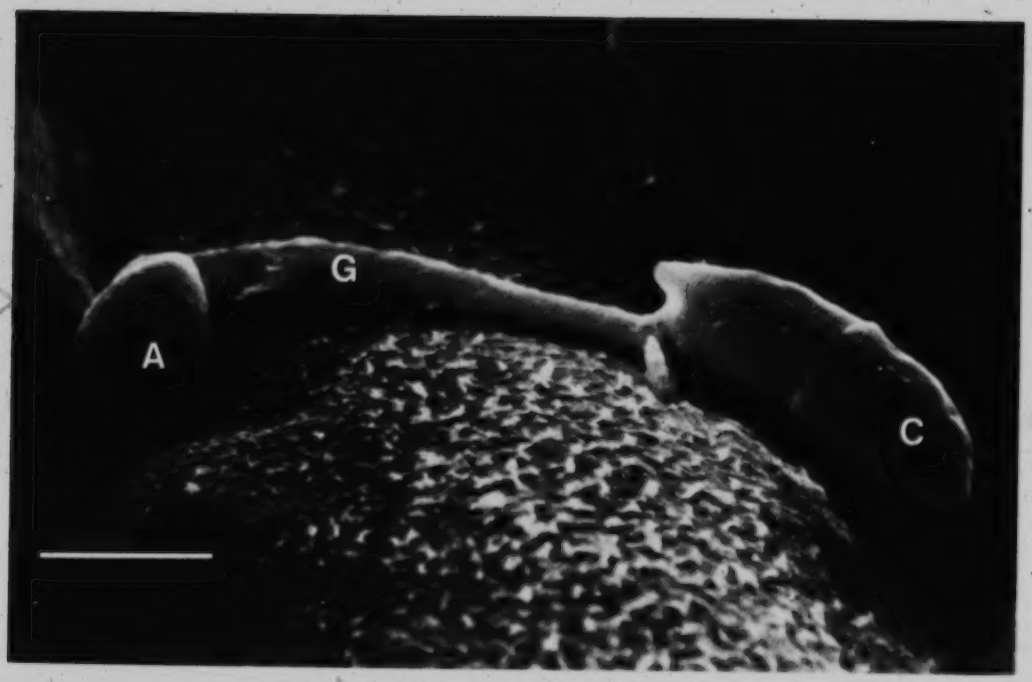


FIG. 1. A conidium, germ tube and appressorium of C. graminicola on the surface of a barley leaf. The bar is 5u long. C = conidium, G = germ tube, A = appressorium.

appressoriation in C. graminicola with 15°C and 35°C being the lower and upper limits respectively. Amino acids containing sulfhydryl groups blocked appressoriation in C. graminicola (Netolitzky and Skoropad 1971). Netolitzky (1969) found that neither carbon:nitrogen ratios nor hydrogen-ion concentration influenced appressoriation while contact with a hard surface stimulated appressoriation.

In this project the following four parts of the prepenetration phase have been studied: (1) the time-course of both germination and appressoriation; (2) factors controlling the location of appressoriation; (3) the possible role of cAMP in the control of appressoriation; and (4) the nature of the adhesive material or mucilage which binds the appressorium to its substrate.

NOTE

This thesis is set up in the format of four separate papers with a general introduction and discussion. The first section, the model of germination and appressoriation, has been published (Lapp and Skoropad 1976). The rest of the data are contained in Appendix I. The other three sections will be submitted for publication at an early date.

SECTION I

A MATHEMATICAL MODEL OF GERMINATION AND APPRESSORIATION

ABSTRACT

A mathematical model, which is a modification of the Gompertz equation, can determine the number of germinations and of appressorial formations in a population of conidia of Colletotrichum graminicola. It will determine both the maximum proportion of conidia which will germinate or which will form appressoria, and the time for these events to occur. The equation parameters which are functions of biotic and abiotic conditions are determined using a nonlinear regression programme. The data used to test the model show that continuous light on the parent fungal colony reduces subsequent conidial germination, but does not change the time taken to reach this maximum.

INTRODUCTION

Although considerable work has been done on germination of conidia and appressoriation, few workers have looked at the time course and fewer still have attempted to fit a mathematical equation to the time - germination curve. Wellman and McCallan (1942) used a modified probit analysis (Finney, 1947) to model germination of spores of Sclerotinia fructicola, Glomerella cingulata, Alternaria solani and Macrosporium sarcinaeforme. Massie and Nelson (1973) used the linear least squares multiple regression technique to model Helminthosporium maydis spore germination. Waggoner

and Parlange (1974a, 1974b, 1975) also used probit analysis to model conidial germination of A. solani. They extended their work to develop a complex box model of germination (Waggoner, 1974), but they stated that "...a chi-square shows that the deviation of the observations from the curve are statistically significant..." (1974a). Wallace et al. (1975) have modelled the germination of Entomophthora aphidia. Neither we nor Emmett and Parberry (1975) found any references to a model of appressoriation.

In this paper we suggest the use of the Gompertz equation as a model of both germination and appressoriation of Colletotrichum graminicola (Ces.) G. W. Wils. This equation was first proposed by Benjamin Gompertz (1825) to predict human mortality and was used to this end by actuaries for many years (King, 1902). The form of the equation used was:

$$y = AB^{r^x} \qquad 1.$$

where y is the mortality at time x, and A, B and r are constants. It depended on the one basic assumption that "...the average exhaustions of a man's power to avoid death were such that at the end of equal infinitely small intervals of time, he lost equal portions of his remaining power to oppose destruction which he had at the commencement of those intervals..." (Gompertz, 1825).

Analagous to the logic of Gompertz, if under favourable germination conditions the spore population loses equal

infinitely small portions of its ability to germinate in equal infinitely small intervals of time, the spore population will germinate in a manner which will be described by the Gompertz function. While this is a reasonable assumption, the use of this or the other germination equations is empirical. The choice depends on the accuracy of the fit of the equation to the data. There is no biological evidence on which to base any particular equation at this time.

The Gompertz function has been used in human biology to model the "rate of aging" (Shock, 1967), extension to humans of animal mortality due to radiation (Sacher, 1956), population growth (Davies, 1927) and growth of individuals (Deming, 1957; Marubini and Resele, 1971). Zoologists have used it to model individual growth of razor clams (Weymouth *et al.* 1931), fish populations (Beverton, 1953; Silliman, 1968), bird growth (Rickleffs, 1973), chick heart growth (Medawar, 1940) and in radiation work (Storer, 1965; Tyler and Stearner, 1966; Dipert and Tyler, 1971). In plant biology, the Gompertz equation has been used to model the growth of *Pelargonium* leaves (Amer and Williams, 1957), growth of corn leaves (Baker *et al.*, 1975), growth of an Avocado graft (Chance and Foerster, 1973), shoot growth of *Hevea brasiliensis* (Lioret, 1974), apple drop (Llewelyn, 1968) and forage yields (Jones and Bartholomew, 1973). The Gompertz equation has also been used outside biology (Peabody, 1924). The general properties of the function and

comparisons of this function with other growth functions have been published (Wright, 1926; Courtis, 1929, 1937; Winsor, 1932; Lumer, 1937; Mather, 1949; Richards, 1959; Silliman, 1969).

THEORETICAL ANALYSIS

The form of the Gompertz equation used in this paper was suggested by Winsor (1932):

$$y = Ke^{-e^{a-bx}} \qquad 2.$$

where y is the number of spores that germinated or have formed appressoria at time x, and K, a and b are constants. The constant K is the maximum number of spores in the population which have germinated or which have developed appressoria and is of obvious biological signifigance. It is difficult to assign direct biological meaning to the constants a and b which interact to define the point of inflection (x=a/b) and in a sense interact to determine the rate. However, using an idea similar to that of Courtis (1929), a and b may be used to define the time taken to reach 99% of the maximum. By substituting y = 0.99K into equation 2 the following is obtained:

$$x_K = \frac{a - \mathrm{Ln}\mathrm{Ln}1.0101}{b} \qquad 3.$$

where Ln is the natural logarithm and x_K is effectively the time taken to reach the maximum and is biologically significant.

MATERIALS AND METHODS

C. graminicola, the causal agent of anthracnose of grasses, is a suitable test fungus since it germinates and forms appressoria well under laboratory conditions. The appressoria are dark, distinctive club-shaped structures. The model has been tested on data accumulated to determine the effect on subsequent germination and appressoriation of different day lengths of low level light on the parent fungal colonies. Netolitzky (1969) found that light was necessary to obtain sporulation with *C. graminicola* cultures. Strong light on the parent cultures reduced the subsequent germination of *C. falcatum* conidia (Singh, 1973). A single spore culture of *C. graminicola* was grown on potato sucrose agar (infusion from 200 g of potatoes, 20 g sucrose, 17 g agar, made up to 1 l with distilled water and dispensed in test tube slants) at 25°C. Light was supplied by a cool white (F15.T12) fluorescent lamp in day lengths of 6, 12, 18 and 24 hours. The incident illumination on the culture tubes as measured by a plant growth photometer (International Light IL150) was 0.7, 0.3, 0.0 u watts/cm² - nanometer in the blue, red and far red bands respectively. Spore suspensions were made after 11 days by flooding the cultures with sterile distilled water and rubbing the colonies gently with a platinum loop. The suspension was filtered through four layers of cheese cloth and diluted to give approximately 90,000 spores/ml by a hemocytometer count.

Clean cover slips were placed in petri dish moist

chambers, 0.3 ml of spore suspension were pipetted on to the cover slips which were then incubated at 25°C in the dark. Every two hours for the next 32 hours five cover slips were removed from the incubator in a preset random pattern and dried. The cover slips were mounted with glycerine and 50 spores from each were scored for germination and appressoriation. The experiment was repeated three times for each light regime. A non-linear regression analysis to the Gompertz equation of each time curve was performed using a FORTRAN subroutine and BMD:07R (Sampson, 1973).

RESULTS AND DISCUSSION

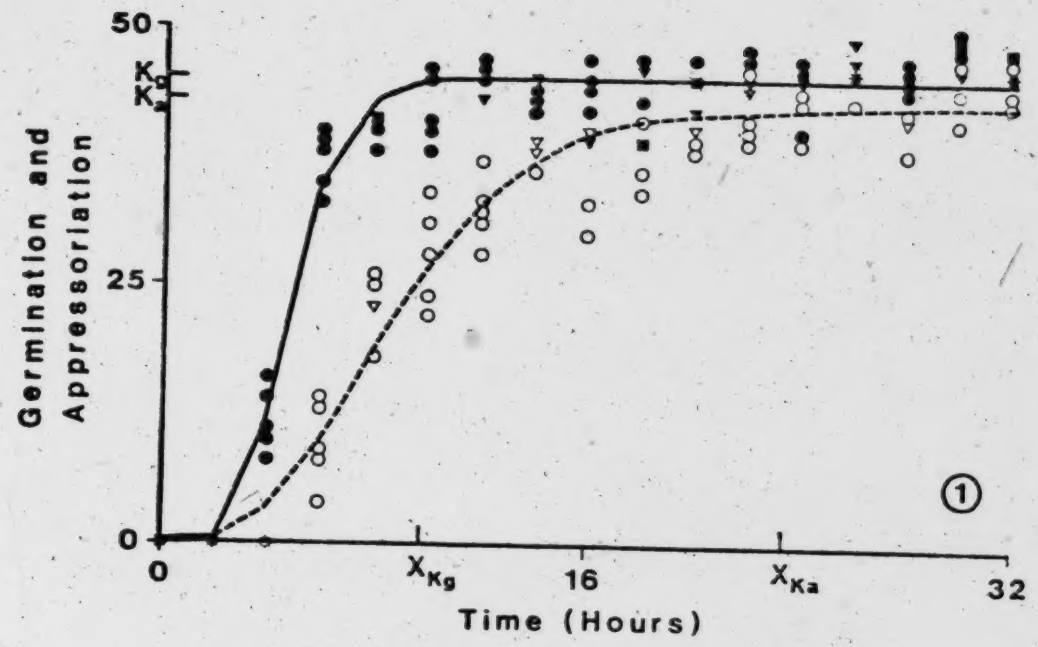
Two examples from the twelve pairs of germination and appressoriation time curves are shown in FIGS. 1 and 2. Both the actual data and the theoretical line produced by the regression of those data to the Gompertz equation are shown. In all cases the fit of the regression line is highly significant ($P<0.0001$). The regression removed 90 - 97% of the variance in the data. Thus the Gompertz equation fits the germination and appressoriation data and allows the reduction of a large mass of data to three parameters (K, a and b) which adequately describe the time curve of germination or appressoriation.

The means of the parameters (K, a and b) plus the calculated parameter (x_K) of the three replicates for each of the four light regimes are shown for germination (Table 1) and appressoriation (Table 2). These means were tested for differences by analysis of variance and Duncan's new

FIG. 1. The results of germination and appressoriation for the conidia collected from the cultures of C. graminicola grown under 12 hours of light are shown here along with the Gompertz regression line. The Gompertz parameters are: K = 45.0, a = 3.63, b = 0.842 and x_K = 9.8 for germination; K = 43.1, a = 2.05, b = 0.283 and x_K = 23.5 for appressoriation.

FIG. 2. The results of germination and appressoriation for the conidia collected from the cultures of C. graminicola grown under continuous light are shown here along with the Gompertz regression line. The Gompertz parameters are: K = 35.1, a = 2.84, b = 0.651 and x_K = 11.4 for germination; K = 33.1, a = 2.46, b = 0.386 and x_K = 18.3 for appressoriation.

KEY: Germination: data points, 1=●, 2=▼, 3=■, 3+=✱ coincident points; regression line ——————.
Appressoriation: data points, 1=○, 2=▽, 3=□, 3+=☼ coincident points; regression line - - - - - - - -. K_g = K for germination, K_a = K for appressoriation, X_g = x_K for germination, X_a = x_K for appressoriation.


50
25
0
Germination and Appressorlation
0
X_{Kg}
16
X_{Ka}
32
Time (Hours)
①

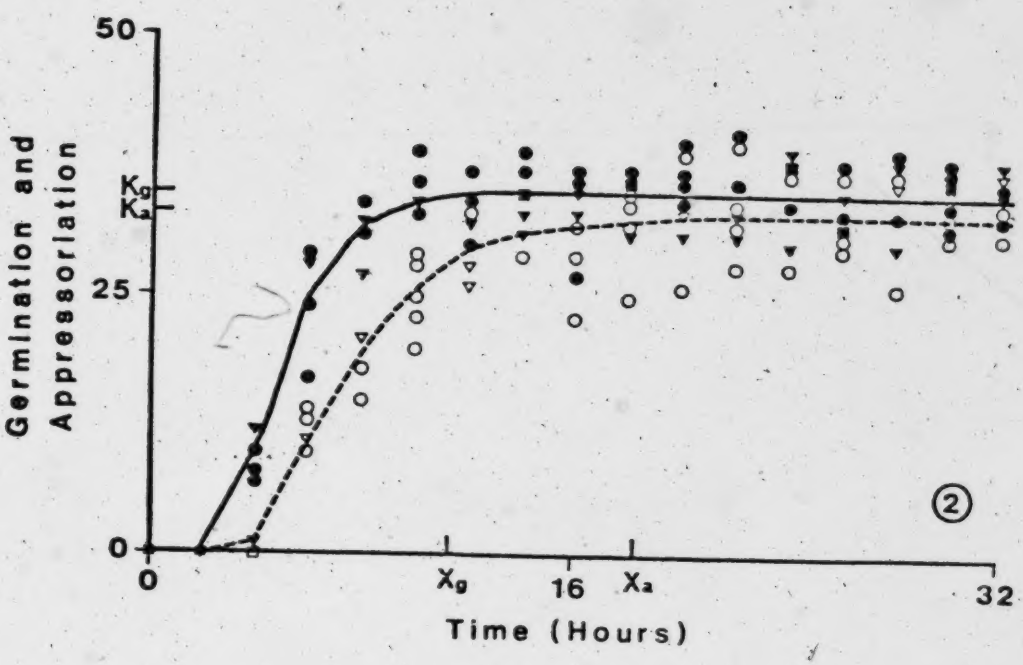

50
25
0
Germination and Appressorlation
0
X_g
16
X_a
32
Time (Hours)
②

TABLE 1.

Mean values of the Gompertz parameters (K,a and b) and the calculated parameter (x_K) for the germination of conidia of C. graminicola for the four daylengths.

Daylength	K	a	b	x_K
6	45.0 f*	2.99 f	0.72 f	10.6 f
12	43.7 f	3.28 f	0.78 f	10.3 f
18	44.1 f	2.44 f	0.66 f	10.9 f
24	36.9 g	2.36 f	0.57 f	16.6 f

* Within columns, numbers followed by a different letter are significantly different (P>0.05) by Duncan's new multiple range test.

multiple range test. There were no significant differences between any of the parameters for the 6, 12 or 18 hour light regimes for germination. Parameter K for 24 hours of light was significantly less (P<0.05) but parameters a and b for 24 hours of light were not significantly different from the other light regimes. Although x_K was larger, this difference was not significant. Thus 24 hours of light reduced the proportion of conidia of C. graminicola which can germinate but did not change the time taken for the maximum number of spores to germinate. Similarly the appressoriation maximum, K, for continuous light is significantly reduced (P<0.05) but the other parameters (a, b and x_K) are not significantly different. There are no significant differences in the

TABLE 2.

Mean values of the Gompertz parameters (K, a and b) and the calculated parameter (x_K) for the appressoriation of germ tubes of conidia of C. graminicola for the four daylengths.

Daylength	K	a	b	x_K
6	42.3 f*	2.02 f	0.25 f	27.3 f
12	42.2 f	2.07 f	0.25 f	33.1 f
18	42.7 f	2.03 f	0.26 f	25.1 f
24	34.6 g	2.02 f	0.31 f	23.4 f

*Within columns, numbers followed by a different letter are significantly different (P>0.05) by Duncan's new multiple range test.

proportions of germinated conidia which form appressoria. Thus the effect of the 24 hour light regime seems to be on germination and not appressoriation.

CONCLUSIONS

The Gompertz equation is a suitable model of the time course of conidial germination and appressoriation in C. graminicola. Other work in this laboratory has shown that this equation also fits the germination time curve of Alternaria brassicae (Lapp and Skoropad, unpublished data). This technique allows the reduction of a large mass of data to three parameters which can be used to describe maximum germination and appressoriation and the time taken to reach these maxima. With the test data we have shown that

continuous low level light on the parent cultures reduces the proportion of conidia which will germinate but does not affect the rate of germination.

SECTION II

LOCATION OF APPRESSORIA ON LEAF SURFACES

ABSTRACT

The location of appressoriation in C. graminicola was studied using natural and artificial (nail polish and epoxy) leaf surfaces. The distribution of appressoria relative to the groove over the anticlinal walls of the epidermal cells was the same for natural and artificial surfaces. This evidence shows that the location of appressoriation is controlled by the host topography and not by localized host exudations.

INTRODUCTION

Appressoriation (appressorial formation) is one of the crucial steps in the successful penetration of the host for many pathogenic fungi, and frequently occurs at a specific location on the surface of the host plant. The appressoria of Colletotrichum graminicola (Ces.) Wils. tend to form most often in the groove at the junction of the anticlinal walls of the epidermal cells (Netolitzky 1969). The germ tubes of Pellicularia filamentosa grow along the junction of the epidermal cells where appressoriation occurs (Flentje 1957). This response is controlled by host chemical exudates and the nature of the surface ie. the cuticle (Kerr and Flentje 1957). Preece et al. (1967) found that 90% and 93% of the appressoria of Erysiphe polygoni and Peronospora parasitica respectively, formed in the junction area over the anticlinal walls of the epidermal cells. Anderson and Walker

(1962) reported about C. orbiculare that "when the spores did not lie over the junction between cells, the germ tubes normally grew to such a juncture prior to appressorial formation". Preferential appressoriation in the groove over the anticlinal walls has been reported in Tubercinia trientalis, Phytophthora omnivora, Protomyces macrospora (De Bary 1887), Dothidella ulei (Blayquez and Owen 1963), and C. lagenarium (Akai et al. 1967).

Contact in vitro by the germ tube with a surface of sufficient hardness has been found to be the most common stimulant of appressoriation (Emmett and Parbery 1975). Early workers reported that contact by the germ tubes of Gloeosporium fructigenum (Hasselbring 1906), Sclerotinia libertiana (Boyle 1921) or C. trifolii (Sampson 1928) with a hard surface led to appressoriation. Contact stimulus in C. lindemuthianum has been reported (Dey 1919) but this response could be blocked by the addition of nutrients (Leach 1923). Using membranes of paraffin, gelatin or other materials, it has been shown that in Botrytis cinerea (Brown and Harvey 1927), Puccinia triticina, P. graminis, P. glumarum (Dickinson 1949), C. phomoides (Van Burgh 1950), and C. graminicola (Netolitzky 1969) contact with a surface of sufficient "hardness" is necessary before appressoriation will occur. In C. gloeosporioides, germ tubes from conidia suspended in a hanging drop formed appressoria only when contact was made with the glass (Dey 1933, Purkayastha and Sen Gupta 1973). Solanki et al. (1974) had similar results

with C. capsici. Parbery (1963) found that contact with a hard surface was necessary for proper appressoriation in a Phyllachora sp. Lack of surface contact led to the formation of abnormal appressoria. Contact stimulus of appressoriation has also been reported in C. atramentarium (Illman et al. 1959), C. dematium, C. trichellum (Sutton 1962), C. truncatium (Staples and Yaniv 1975) and Pythium irregulare (Agnihotri 1969).

In G. fructigenum (Hasselbring 1906), C. lindemuthianum (Leach 1923), C. phomoides (Van Burgh 1950) and C. piperatum (Grover 1971) the addition of nutrients to the infection drop inhibited appressoriation, while in C. gloeosporioides additional nutrients enhanced appressoriation (Purkayastha and Sen Gupta 1973). Host-plant exudates stimulated the formation of infection structures in P. filamentosa (Kerr and Flentje 1957), Thanatephorus cucumeris (Flentje et al. 1963) and C. piperatum (Grover 1971). Appressoriation was stimulated by cuticular material in Alternaria porri (Akai et al. 1967) and Erysiphe graminis (Yang and Ellingboe 1972). Several sugars and a few organic nitrogen sources enhanced appressoriation in C. capsici (Solanki et al. 1974). Four phenolic related compounds blocked appressoriation in C. falcatum (Srinivasan and Norasimhan 1971). Netolitzky (1969) found that in C. graminicola some nutrients stimulated appressoriation while amino acids containing sulfhydryl groups blocked appressoriation (Netolitzky and Skoropad 1971). Thus generally the

controlled location of appressoriation has been attributed to the effect of the "contact stimulus" at the anticlinal groove on the germ tube.

Preliminary work with the scanning electron microscope (SEM) suggested that appressoriation in C. graminicola does not occur right at the bottom of the anticlinal groove but near to it. If this observation was correct then it would be unlikely that blocking of the germ tube growth by the anticlinal groove would be the stimulator of appressoriation. Alternatively, it is reasonable to suggest that the host-plant cuticle surface is chemically or physically different near the anticlinal groove and that this difference is the stimulator of appressoriation. These hypotheses were tested by germinating conidia of C. graminicola on natural and on artificial leaf surfaces and determining the distribution of appressoria on these surfaces.

MATERIALS AND METHODS

For investigations on natural leaf surfaces, 8 cm. pieces were cut from the center of the second leaves of 10 day old Parkland barley (CAN 210) plants (grown at 21 C in soil with 16 hr. of light in a controlled environment chamber) and floated on a solution of 10 ppm aureomycin and 40 ppm benzimidizole (Person et al. 1957, Netolitzky 1969). For artificial leaf surfaces, negative molds were made from similar intact leaves with silicone rubber (Liquid silicone rubber, RTV11, General Electric). After curing for 4

minutes, the negative replicas were carefully stripped off the leaves and positive replicas of clear nail polish or epoxy (Araldite 506, E. F. Fullam, Inc.) were made. A nail polish replica was made first and then an epoxy replica was made from each negative silicone replica. The positive replicas were washed with soap and water and rinsed 3 times in distilled water before use. One epoxy replica was coated with carbon gold (15-20 nm. thick). The replicas were placed in petri dish moist chambers.

C. graminicola, originating from a single spore isolate from oats grown near Edmonton, Alberta, was grown on potato sucrose agar (infusion from 200 g of potatoes, 20 g sucrose, 17 g agar, made up to 1 litre with distilled water and dispensed in test tube slants) at 25 C with 12 hr light from a cool-white fluorescent lamp. Spore suspensions were made after 11 days by flooding the cultures with sterile distilled water and rubbing the colonies gently with a platinum loop. The suspension was filtered through four layers of cheesecloth and diluted to give about 90,000 spores/ml by a hemacytometer count. Small drops of the conidial suspension were pipetted onto the natural and artificial leaf surfaces which were then incubated for 30 hr at 25°C in the dark.

Growth was stopped on the natural leaves by freezing them in liquid freon, followed by freeze-drying at -70°C (Edwards Pearse Tissue Dryer) in preparation for observation by the SEM (Cambridge Stereoscan S4). Excess water was

decanted off the artificial surfaces which were then dried over anhydrous $CaSO_4$. Both natural and artificial leaf surfaces were coated with carbon-gold (15-20 nm. thick) and photographic mosaics of the infected areas were made with the SEM, keeping the incident angle as close to 90° to the surface as possible.

The distance from each appressorium (in each photographic mosaic) to the nearest groove was measured using vernier calipers. Because the distributions of appressoria to groove distances resembled a Poisson distribution, a square root transformation was done on the data before analysis of variance was used to test the differences between treatments.

RESULTS AND DISCUSSION

There was little difference in the shape of the distributions of appressoria on the surface of natural leaf (FIG. 1), nail polish (FIG. 2), epoxy (FIG 3.) and carbon-gold coated epoxy (FIG. 4). Analysis of variance and Duncan's new multiple range test on the transformed (square root) data showed that in two of the treatments the mean appressorium-to-groove distance was significantly ($P=0.05$) smaller (Table 1). Both these treatments were natural leaf surfaces and were significantly different from other natural leaf surfaces. No differences were detected between the other natural or artificial surfaces. Because there was no difference between the nail polish or epoxy replicas nor the gold-carbon coated replica, it is unlikely that there was

FIGS. 1-4. Distributions of the distances from appressoria of C. graminicola to the nearest groove over the anticlinal walls of the epidermal cells on natural leaf surfaces and artificial leaf surfaces. Percent of the total number of appressoria are plotted against the distance broken into 1.5 μ increments. FIG. 1. Natural leaf surface. FIG. 2. Nail polish replicas. FIG. 3. Epoxy replicas. FIG. 4. Carbon-gold coated epoxy replicas.

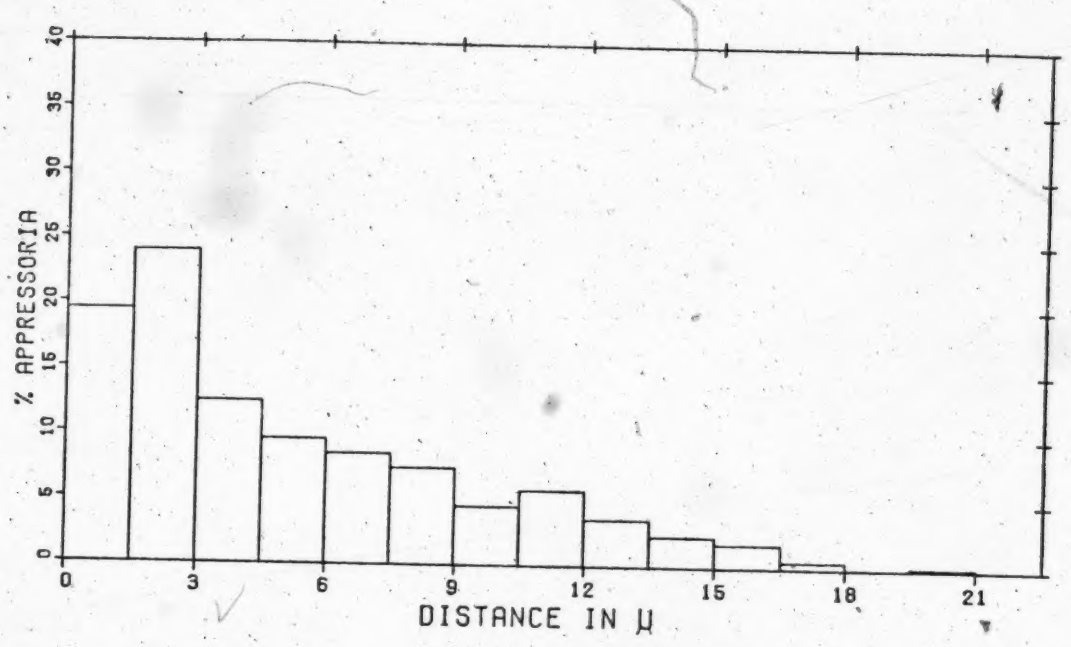


FIG. 1. NATURAL LEAF SURFACE

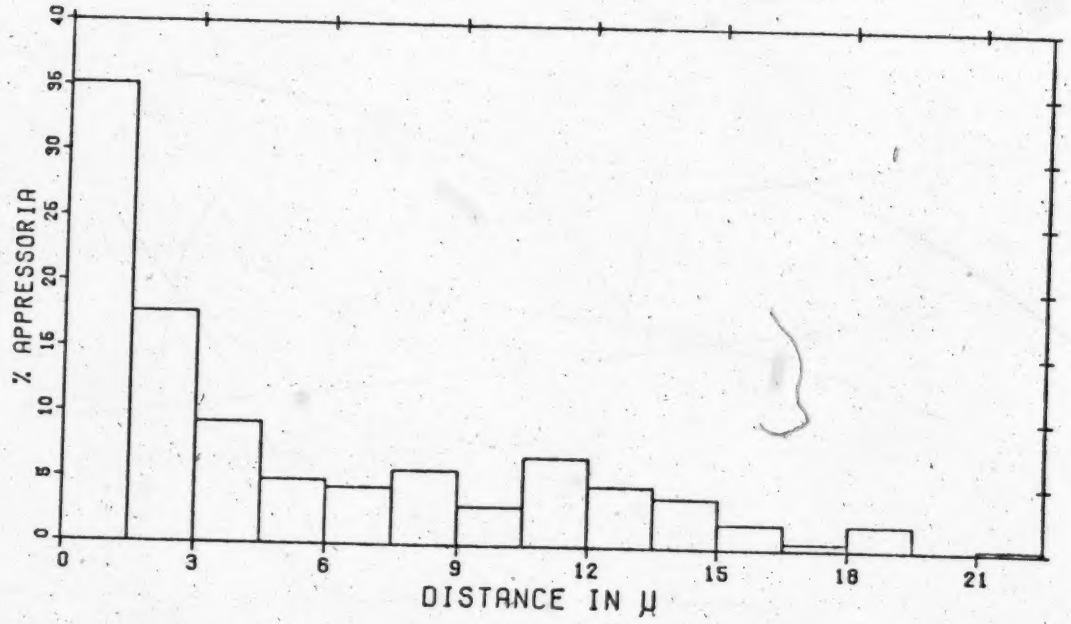


FIG. 2. ARTIFICIAL (NAIL POLISH) LEAF SURFACE

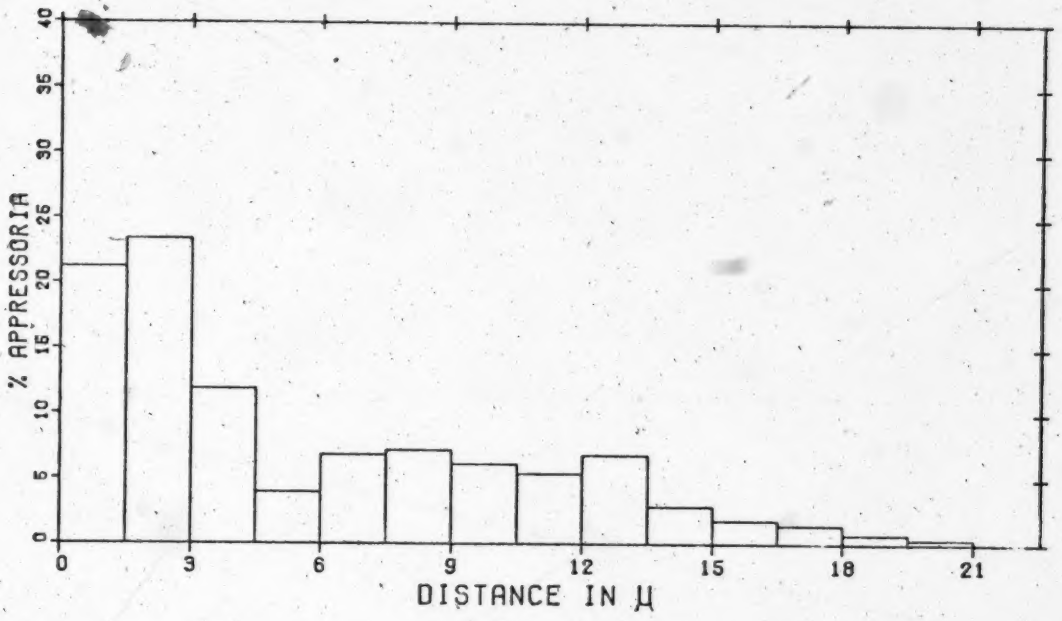


FIG. 3. ARTIFICIAL (EPOXY) LEAF SURFACE

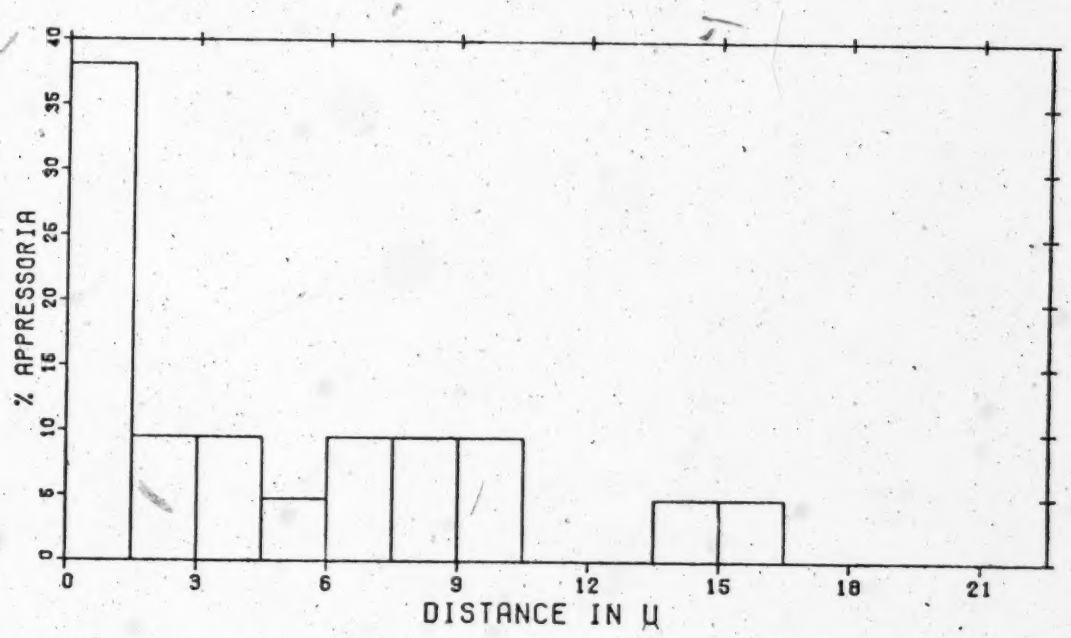


FIG. 4. ARTIFICIAL (CARBON-GOLD) LEAF SURFACE

TABLE 1

Means of the distances in μ from the appressorium of C. graminicola to the nearest epidermal groove for different leaf surfaces. The sample sizes are also shown.

SURFACE	MEAN in μ	SAMPLE SIZE
LEAF	1.72 a[1]	53
LEAF	2.86 ab	46
LEAF	3.77 bc	16
NAIL POLISH	3.83 bc	315
GOLD CARBON	4.11 bc	21
NAIL POLISH	4.14 bc	19
EPOXY	4.16 bc	36
NAIL POLISH	4.47 bc	33
LEAF	4.61 c	222
EPOXY	4.68 c	179
EPOXY	4.71 c	63
LEAF	5.34 c	218

1. Means followed by the same letter are not significantly different (P=0.05) by Duncan's new multiple range test.

any chemical carry-over from the natural leaves. Thus it is concluded that the location of appressoriation is dependent on the physical surface structure of the leaf and is not dependent on any local chemical difference in the host cuticle.

Most of the appressoria form sufficiently far from the

groove (FIGS. 1-4) so that it seems unlikely that impeding of the germ tube growth by the groove is the major stimulant of appressoriation. Dickinson (1964, 1969, 1970) in detailed studies of the response of Puccinia spp. germ tubes to artificial membranes, found that the rust germ tubes could recognize particles about the size of cellulose molecules (120 x 1.2 nm.). However, due to the rough nature of the outer layer of the barley-leaf surface, it seems unlikely that the germ tube of C. graminicola would be significantly influenced by oriented structures of this size (120 x 1.2 nm).

Wynn (1976), in similar work with Uromyces phaseoli, on leaf replicas found that the germ tube could recognize both the leaf ridges and the stomatal lips. This control was through the leaf topography and not due to any host-chemical stimulus. Appressoriation is primarily controlled by the fungal genotype (Emmett and Parbery 1975) and in C. graminicola the expression of this genotype appears to be controlled by the topography of the host-leaf surface.

SECTION III

EFFECT OF CYCLIC AMP ON APPRESSORIATION

ABSTRACT

Regulation of appressoria formation in Colletotrichum graminicola (Ces.) Wils. was investigated using exogenous adenosine 3':5'-cyclic monophosphate (cAMP), adenosine 5'-monophosphate (AMP), 1,3-dimethylxanthine (theophylline) and sodium fluoride (NaF). Each of these chemicals inhibited appressoriation but only NaF inhibited germination. Increased levels of cAMP have no effect on germination of the conidia but inhibit appressoriation.

INTRODUCTION

The causes of appressorial formation (appressoriation) have long been a subject of investigation. Appressoriation has been attributed most commonly to the germination tube striking a surface of sufficient hardness (Hasselbring 1906; Dey 1919; Emmett and Parbery 1975). However easily this may be demonstrated in the laboratory, it does nothing to explain how surface hardness induces appressoriation.

A number of chemicals have been tested for their effect on appressoriation. Various sugars and nitrogen sources have been tested for this effect in Colletotrichum capsici (Solanki et al. 1974), C. gloeosporioides (Purkayastha and Sen Gupta 1973) and C. graminicola (Netolitzky 1969). Compounds containing sulfhydryl groups appeared to be the only ones that consistently inhibited appressoriation

(Netolitzky and Skoropad 1971). This was attributed to the cell-division blocking properties of sulfhydryl groups.

The effect of several metabolic inhibitors on germination and appressoriation in C. trifolii (Miehle and Lukezic 1972) and C. truncatum (Staples and Yaniv 1975) have been investigated. Both cycloheximide and actinomycin blocked germination, but if applied after germination, did not block appressoriation. However, 5-fluorodeoxyuridine did not block germination but prevented appressoriation. Staples and Yaniv (1975) concluded that DNA synthesis is necessary for appressoriation but not germination and that protein synthesis early in germination is needed for appressoriation. Part of this information needs reassessment since cycloheximide is not a specific inhibitor of protein synthesis in vivo (McMahon 1975).

Ever since its discovery (Cook et al. 1957; Sutherland and Rall 1957), adenosine 3':5'-cyclic monophosphate (cAMP) has been implicated in the regulation of a wide variety of cellular functions (Bitensky and Gorman 1973). The conversion of adenosine triphosphate (ATP) to cAMP is catalysed by the enzyme adenyl cyclase which is bound predominantly to the plasma membrane (Perkins 1973).

Limited information is available on cAMP systems in fungi. Growth habit and mycelial and conidial characteristics in Neurospora crassa respond to cAMP levels (Terenzi et al. 1974; Scott and Solomon 1975). Yeast-mycelium dimorphism in Mucor racemosus was reported to be

controlled by cAMP (Larsen and Sypherd 1974). Cyclic AMP levels were found to change during differentiation in Blastocladiella emersonii (Silverman and Epstein 1975). Galsky et al. (1972) reported that cAMP controls the perithecial-initial formation in Monacrosporium doedyciodes and Feldman and Thayer (1974) found that cAMP derepresses tyrosinase production in N. crassa, an enzyme involved in sexual differentiation. Uno and Ishikawa (1973a,b) found possible participation by cAMP in inducing fruiting in Coprinus macrorhizus. Cyclic AMP inhibited growth in Puccinia graminis tritici (Bose and Shaw 1974), increased the rate and frequency of chlamydospore production in Fusarium solani (Meyers and Cook 1972), enhanced the production of the sex regulating substance, zearalenone, in F. roseum (Wolf and Mirocha 1974) and promoted aggregation of conidia and germlings in Aspergillus niger (Wold and Suzuki 1973).

In the cellular slime molds, cAMP is an acrasin which regulates aggregation (Gross 1975) and also controls differentiation of the stalk cells (Bitensky and Gorman 1973).

While evidence on the presence of cAMP in higher plants is still inconclusive (Lin 1974; Bressan et al. 1976), its presence in fungi is proven (Flawia and Torres 1972a, b, 1973; Wold and Suzuki 1974). This paper reports the effect

of cAMP on conidial germination and appressoriation in C. graminicola, the causal agent of anthracnose of cereals and other grasses.

MATERIALS AND METHODS

C. graminicola was isolated from stems of common oats (Avena sativa) collected near Edmonton, Alberta, Canada. Suspensions of conidia were made in sterile distilled water from 11-day-old cultures grown on potato sucrose agar test tube slants (infusion from 200 g of potatoes, 20 g sucrose, 17 g agar, made up to 1 l with distilled water) in 18 h of light from a cool white fluorescent bulb at 25° C. The conidial suspensions were filtered through four layers of cheese cloth. Solutions of adenosine 3':5'-cyclic monophosphoric acid, sodium salt (Sigma); adenosine 5'-monophosphoric acid, Type II, sodium salt (AMP, Sigma); sodium fluoride (NaF, J. T. Baker) and 1,3-dimethylxanthine (theophylline, Sigma) were made up with the conidial suspension and suitably diluted with the spore suspension in order to maintain a concentration of about 90,000 conidia per ml. In all experiments spore suspensions in sterile distilled water were used as controls. Clean cover slips were placed in petri dish moist chambers; approximately 0.3 ml of the spore suspensions were pipetted onto the cover slips and these preparations were incubated at 25° C in the dark. After 24 h, the cover slips were dried and mounted on slides with glycerin.

The conidia were observed using phase contrast optics,

and 250 spores for each treatment were scored for germination and appressoriation. The experiment was done a total of three times for each compound.

RESULTS AND DISCUSSION

Exogenous cAMP (Fig. 1), AMP (Fig. 2) and theophylline (Fig. 3) inhibited appressoriation but not germination. Both germination and appressoriation were inhibited by NaF (Fig. 4). If the decrease in appressoriation by NaF was due solely to the reduction in germination, a plot of the differences of appressoriation from germination would be a straight line. But this graph (Fig. 5) shows a peak and thus it is concluded that appressoriation is more sensitive than germination to NaF. Probit analysis (Finney 1947) was attempted but due to the two step curve which the data seem to follow, the fit of the probit curve was not acceptable. However, correlation analysis was done with the chemical concentrations in millimoles versus the reduction in number of appressoria from the control. There was a significant correlation between dose and response of appressoriation with cAMP, AMP and theophylline and of germination with NaF (Table 1). That the correlation between dose of NaF and inhibition of appressoriation was not significant is probably due to the two-step shape of the data curve. It is concluded that the inhibition of appressoriation is proportional to the level of each of the four compounds.

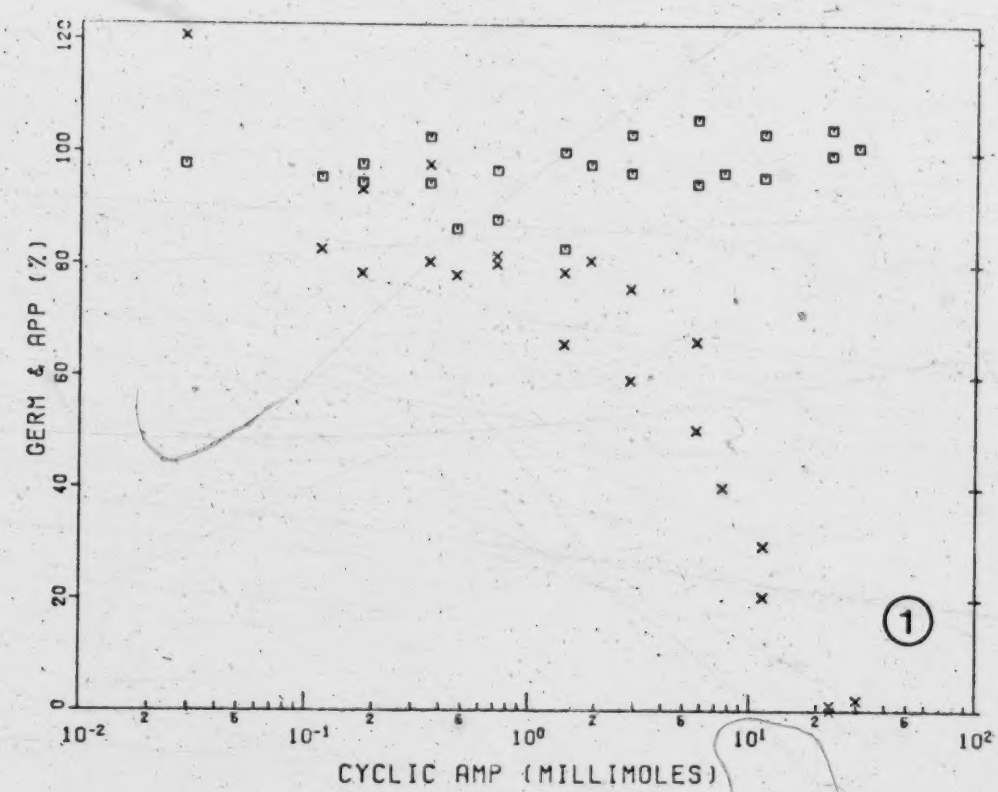
The inhibition of germination by NaF is not surprising since fluoride (10 mM) inhibits 30-70 per cent of adenosine

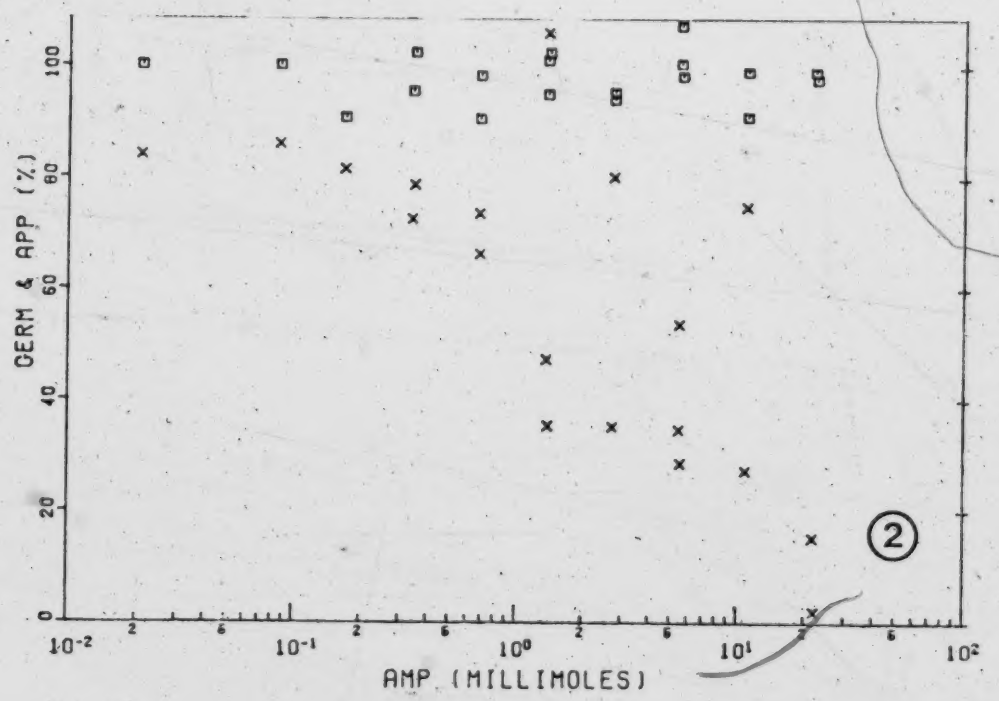
FIGS. 1-4. Plots of the chemical concentrations in log mM versus germination and appressoriation in C. graminicola as % of control. FIG. 1. cAMP. FIG. 2. AMP. FIG. 3. Theophylline. FIG. 4. NaF.

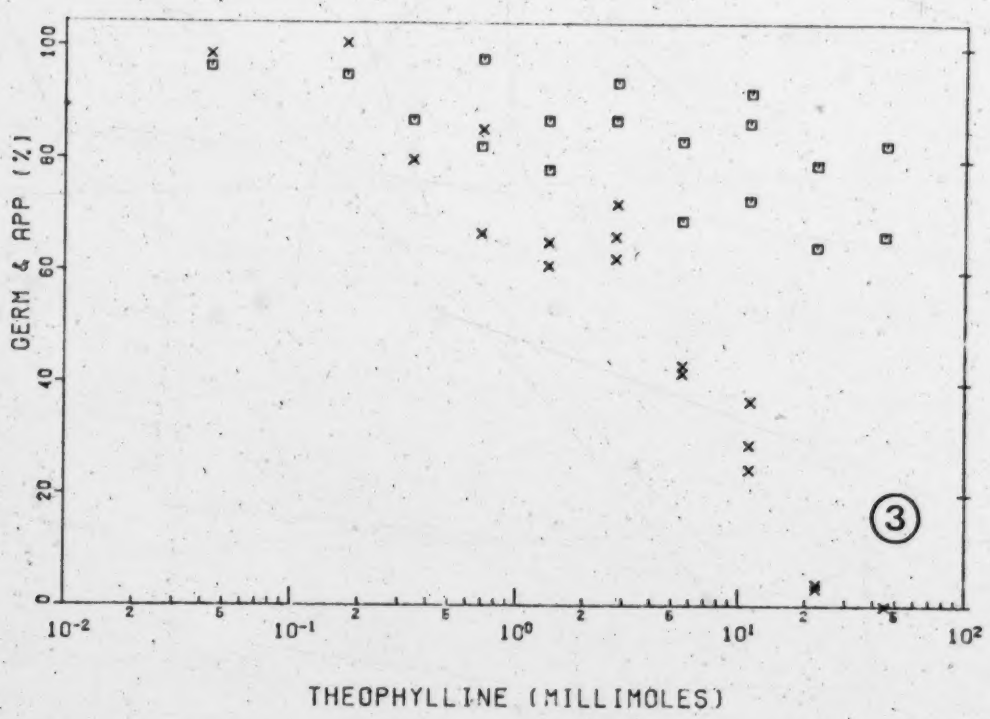
FIG. 5. A plot of concentration of NaF in log mM with the differences between germination and appressoriation in C. graminicola as % of the differences of the controls.

KEY. APP = appressoriation = x , GERM = germination = □ , GERM-APP = difference between germination and appressoriation = ⊙ .

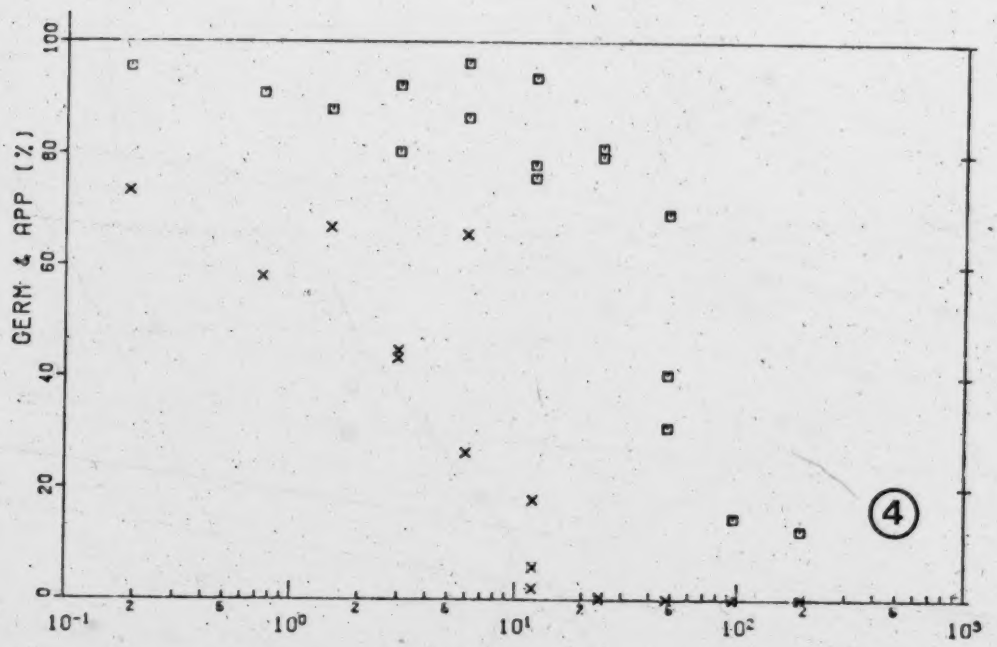
NOTE. The results of the three trials are combined in all the graphs.


GERM & APP (%)
0
20
40
60
80
100
120
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
CYCLIC AMP (MILLIMOLES)
1


GERM & APP (%)
0
20
40
60
100
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
AMP (MILLIMOLES)
2



THEOPHYLLINE (MILLIMOLES)



NAF (MILLIMOLES)

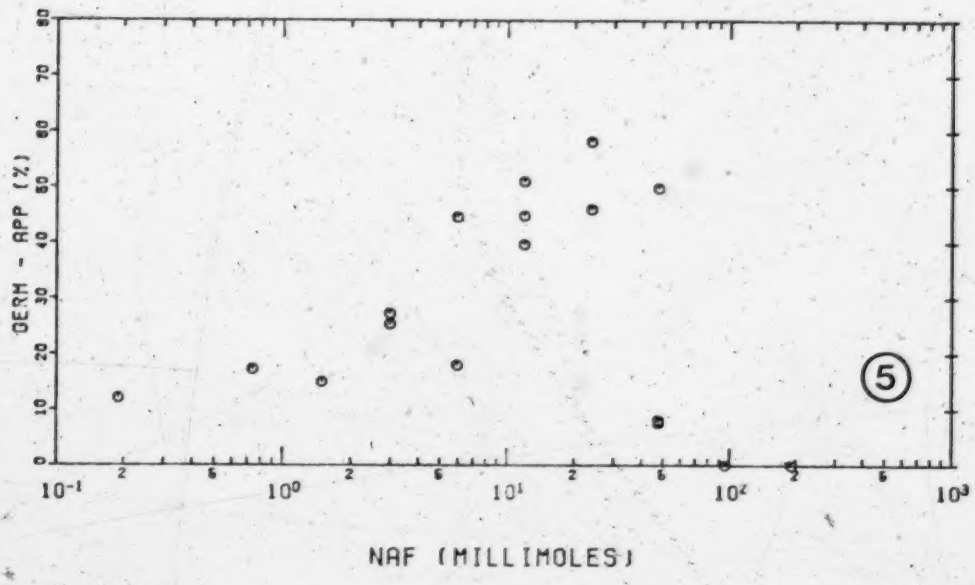

GERM - APP (%)
0
10
20
30
40
50
60
70
80
10^{-1}
2
5
10^{0}
2
5
10^{1}
2
5
10^{2}
2
5
10^{3}
NAF (MILLIMOLES)
⑤

TABLE 1

The correlations of the doses of the four chemicals with the response of germination and appressoriation in C. graminicola for the three replicates.

COMPOUND	CORRELATION COEFFICIENTS					
	GERMINATION			APPRESSORIATION		
cAMP	--[1]	--	--	0.95	0.96	0.94
AMP	--	--	--	0.37[2]	0.80	0.82
THEOPHYLLINE	--	--	--	0.97	0.89	0.92
NaF	0.95	0.91	0.92	0.90[2]	0.74[2]	0.76[2]

1. No inhibition.
2. These correlations are not significant at the 95% level.

triphosphatase (ATPase) activity (Bar 1974). In Allomyces javanicus and Brevilegnia unisperma glucose metabolism is strongly inhibited by fluoride (Ji and Dayal 1971). Alpha-amylase (Allam and Khalil, 1973), enolase, phosphoglucomutase and some metallic-enzymes (Kumar et al. 1972) in Aspergillus niger are inhibited by NaF. However, adenyl cyclase activity is significantly stimulated by NaF (Perkins 1973).

Addition of cAMP to the medium in which conidia of C. graminicola are germinating leads to an inhibition of appressoriation. Treatment with theophylline inhibits appressoriation. Theophylline is known to inhibit 3':5'-

cyclic nucleotide phosphodiesterase that hydrolyses cAMP to AMP (leading to an accumulation of cAMP) (Appleman et al. 1973). Appressoriation is reduced by NaF which stimulates the activity of adenyl cyclase from most organisms. However, so far NaF is assumed not to activate adenyl cyclase in intact cells (Perkins 1973). Also, adenyl cyclase from N. crassa is not activated by NaF (Flawia and Torres 1972a). Inhibition of appressoriation by AMP, among other factors, may be due to increased cAMP through elevated levels of ATP.

The inhibition of appressoriation by each of the four compounds is proportional to the applied concentrations. It is concluded that high levels of cAMP do not affect germination but do inhibit appressoriation. Although the actual levels of cAMP in C. graminicola have not been measured at this time, it appears that in vivo cAMP is involved in the regulation of appressoriation.

SECTION IV

APPRESSORIAL ADHESION

ABSTRACT

The nature of the adhesive "mucilage" of the appressoria of C. graminicola was studied by testing the ability of NH oxalate, NaOH or hemicellulase to release appressoria from glass cover slips on which they had formed. Because only NaOH or hemicellulase released appressoria, it is concluded that the adhesive factor is a hemicellulose.

INTRODUCTION

Appressoria are firmly attached to their substrate (Emmett and Parbery 1975). The appressoria of Gloeosporium fructigenum (Hasselbring 1906) and Colletotrichum graminicola (Ces.) Wils. (Skoropad 1967) can not be washed off with a jet of water from the glass slides on which they had formed. Skoropad (1967) also reported that the appressoria of C. graminicola were not removed from leaves by treatment with absolute alcohol, glacial acetic acid or boiling lactophenol. However the appressoria of C. gloeosporioides can be removed from glass by scrubbing with a detergent (Brown 1975).

The appressorium of C. lindemuthianum has a gelatinous sheath which attaches it to the leaf surface (Dey 1919, Leach 1923). A mucilaginous layer has been reported to be the adhesive material for the appressoria of C. gloeosporioides (Marks et al. 1965), C. lagenarium (Akai et al. 1967) and C. piperatum (Grover 1971). A mucilaginous

sheath surrounding the appressoria has also been found in Sclerotinia libertiana (Boyle 1921), Pellicularia filamentosa (Flentje 1957) and Dothidella ulei (Blayquez and Owen 1963). Other theories have been presented to account for the attachment of an appressorium to its host, but it is now considered that there is a binding substance referred to as "mucilage" (Emmett and Parbery 1975).

Because hemicellulose is considered the universal cement in the plant kingdom (Ehrenthal et al. 1954), it is reasonable to suggest that this "mucilage" is a hemicellulose. Since the appressoria of C. graminicola attach themselves firmly to a glass surface, it is possible to work on the "mucilage" free of interference from any host materials. It is possible to test this theory by the success or failure of materials, which would dissolve or break down hemicellulose, in removing appressoria from glass cover slips.

MATERIALS AND METHODS

Cultures of C. graminicola from a single spore isolate from oats, found near Edmonton, Alberta, were grown on potato sucrose agar (infusion from 200 g of potatoes, 20 g sucrose, 17 g agar, made up to 1 litre with distilled water and dispensed in test tube slants) at 25 C with 18 hr. of light from a cool-white fluorescent lamp. Spore suspensions were made after 11 days by flooding the cultures with sterile distilled water and rubbing the colonies gently with a platinum loop. The suspension was filtered through four

layers of cheesecloth and diluted to give about 90,000 spores/ml by a hemacytometer count. Small drops (0.3 ml) of the conidial suspension were pipetted onto clean glass cover slips. These were incubated in petri-dish moist chambers at 25°C in the dark. After 24 hr., the excess liquid was decanted and the cover slips were dried in an oven at 80°C for eight hours. The cover slips with attached appressoria were stored at room temperature in clean dry petri dishes until needed.

The carbohydrate extraction procedure described by Jensen (1962) was used. The cover slips with attached appressoria were immersed in 0.5% NH_4 oxalate at 75°C, or 4% NaOH at room temperature or 0.5% NH_4 oxalate at 75°C followed by transfer to 4% NaOH at room temperature. Distilled water controls were used throughout.

The ability of hemicellulase (Nutritional Biochemical Corp.) to release appressoria from cover slips was also tested. The hemicellulase was dissolved (0.5%) in 0.2 M phosphate buffer at pH 7.0. Cover slips with attached appressoria were placed in dishes of this solution on a very slow shaker table at room temperature. Phosphate buffer was used for the controls. Activity of the hemicellulase was tested by its ability to lower the viscosity of a solution of carboxymethyl cellulose (Dekker and Richards 1975).

After appropriate times the cover slips were removed from the solutions. They were washed over a millipore filter apparatus and the wash water was run through a 0.45 u filter

to collect any appressoria which had been removed. The remaining test solutions were also filtered. The cover slips and filters were dried over $CaSO_4$. The cover slips were mounted on microscope slides with glycerine while the filters were cleared and mounted with immersion oil. A transect of each cover slip or filter was made using a 40X phase contrast objective and the vernier of a mechanical stage. The length of the transect and the number of appressoria were recorded.

Prior to treatment the exact locations of several appressoria on a few cover slips were recorded using the verniers on the mechanical stage. After treatment the cover slips were processed with the periodic acid-Schiff's (PAS) procedure (Jensen 1962) and the same locations were searched with a microscope. Also using the PAS procedure, appressoria, before and after removal from the cover slips, were compared. Each experiment was repeated at least twice.

RESULTS AND DISCUSSION

Treatment with NH_4 oxalate released very few appressoria from the cover slips (TABLE 1). Treatment with NaOH (TABLE 2) or NH_4 oxalate followed by NaOH (TABLE 3) released all of the appressoria. Very few appressoria were released in the distilled water controls. The hemicellulase released nearly all of the appressoria while the buffer control solution released only a few (TABLE 4). Filtering of the extracting solutions revealed many appressoria in the NaOH and hemicellulase solutions but few if any in the other

TABLE 1

Effect of NH_4 oxalate on the release of appressoria of C. graminicola from the glass cover slips on which they had formed.

REPLICATE	APPRESSORIA / mm²					
	NH_4 OXALATE		CONTROL		EXTRACTING FLUID	
	COVER SLIP	FILTER	COVER SLIP	FILTER	NH_4 OXALATE FILTER	CONTROL FILTER
1	7.16	0.00	13.22	0.00	0.00	0.00
2	5.71	0.27	7.00	0.00		
3	6.82	0.64	8.82	0.10		
4	6.46	0.10	11.68	0.22		
MEAN	6.54	0.25	10.18	0.08		
ST. DEV.	0.62	0.28	2.80	0.10		

extracting fluids (TABLES 1-4). After treatment with NaOH, no residue was seen with the PAS staining procedure nor with phase contrast optics in the places where appressoria had been located. The appressorial pore areas of pre- and post-NaOH treated appressoria appeared the same by both PAS staining and phase contrast optics.

TABLE 2

Effect of NaOH on the release of appressoria of C. graminicola from the glass cover slips on which they had formed.

REPLICATE	APPRESSORIA / mm²					
	NaOH		CONTROL		EXTRACTING FLUID	
	COVER SLIP	FILTER	COVER SLIP	FILTER	NaOH FILTER	CONTROL FILTER
1	0.00	13.00	4.61	0.00	20.05	0.00
2	0.00	8.13	6.34	0.00		
3	0.00	10.85	8.18	0.00		
4	0.00	3.44	5.68	0.00		
MEAN	0.00	8.86	6.20	0.00		
ST. DEV.	0.00	4.12	2.60	0.00		

Ammonium oxalate extracts pectic materials while NaOH extracts hemicelluloses (Jensen 1962). Hemicellulose is defined as that carbohydrate which is soluble in dilute alkali (Aspinall 1970). Because the NH_4 oxalate did not release appressoria from the cover slips, the "mucilage" is not a pectin. It is concluded that the NaOH or hemicellulase was acting on the material which binds the appressoria to

TABLE 3

Effect of NH_4 oxalate followed by NaOH on the release of appressoria of C. graminicola from the glass cover slips on which they had formed.

REPLICATE	APPRESSORIA / mm²					
	NH_4 OXALATE THEN NaOH		CONTROL		EXTRACTING FLUID	
	COVER SLIP	FILTER	COVER SLIP	FILTER	NH_4 OXALATE THEN NaOH FILTER	CONTROL FILTER
1	0.00	0.61	7.93	0.00	15.91	0.00
2	0.00	1.82	8.34	0.00		
3	0.00	1.23	10.61	0.04		
4	0.00	0.43	4.95	0.00		
MEAN	0.00	1.02	7.96	0.01		
ST. DEV.	0.00	0.63	2.33	0.02		

the cover slips and that this adhesive material or "mucilage" is a hemicellulose.

In C. graminicola there is a very small amount of adhesive material because no obvious "mucilage" is discernible with the PAS technique or phase contrast optics

TABLE 4

Effect of hemicellulase on the release of appressoria of C. graminicola from the glass cover slips on which they had formed.

REPLICATE	APPRESSORIA / mm²					
	HEMICELLULASE		CONTROL		EXTRACTING FLUID	
	COVER SLIP	FILTER	COVER SLIP	FILTER	HEMI-CELLULASE FILTER	CONTROL FILTER
1	0.91	6.41	16.66	2.09	7.41	0.20
2	0.82	8.43	15.75	0.66		
3	0.55	10.43	13.43	0.41		
4	0.86	6.02	13.23	1.16		
MEAN	0.79	7.82	14.77	1.08		
ST. DEV.	0.16	2.03	1.70	0.74		

nor in scanning electron micrographs.

Commercial hemicellulases are mixtures of glucanases, xylanases, mannanases and others (Sinner et al. 1975), so it is not possible to be more specific about the nature of this "mucilage". It seems likely that the "mucilage" is a glucan and not a xylan since no xylose was found in the conidial or

hyphal walls of C. lagenarium (Aurial 1974). Both Akai et al. (1967) with C. lagenarium and Netolitzky (1969) with C. graminicola found that the host epidermal cells responded during appressoriation prior to penetration. A glucan from C. lindemuthianum has been found to induce phytoalexin production by the host (Anderson-Prouty and Albersheim 1975). It is possible that hemicellulosic "mucilage", the inducer of the epidermal cell response and the phytoalexin elicitor are related.

GENERAL DISCUSSION

The mathematical model of germination and appressoriation has at least two general applications in plant pathology. In models or simulations of plant disease epidemics, the germination and/or appressoriation model can be used to describe that portion of the simulation which predicts the percent of spores which germinate and/or which form appressoria. The three Gompertz' parameters will vary with changes in the environment eg. temperature. That is to say, K, a and b will be functions of temperature, light, humidity and other factors. If we use temperature as one example, then from the work of Skoropad (1967) we know that the temperature limits of C. graminicola for germination are 10 and 40 C. Thus we can predict that the temperature curve for K for germination will be dome-shaped with a peak at about 25°C and reaching zero at 10 and 40°C. Similarly, the temperature function of K for appressoriation will be dome shaped with a peak between 25-30°C and reaching zero at 15 and 35°C. At the moment we have no information on what the temperature functions of a and b will be. Similar functions will exist for other environmental factors. All these functions can be determined and thus the Gompertz model can be predictive for use in epidemiological simulations.

The Gompertz model's major value in fungal physiology is in the reduction of large masses of data to three easily handled parameters. Comparisons between treatments can easily be made using the three parameters. More information

is obtained using the Gompertz model because both the maximum germination and the time to reach this maximum is available. It is of great interest to know whether a given treatment actually slows appressoriation or only decreases the proportion of appressoriation in the population or both. Information of this kind will add knowledge about how and even where and when a given treatment is operating. Determination of eight to ten data points, 2 in the lag period, 3-4 on the slope and 3-4 on the upper asymptote, will allow establishment of the Gompertz parameters for a given treatment. Thus for the same amount of work, much more information can be ascertained about the effect of a given treatment on germination and/or appressoriation.

In the section on appressorial location it has been shown that the location of appressoria near the groove over the anticlinal walls of the epidermal cells is a function of the physical surface of the host and not of any host exuded chemicals. There are a number of other factors which affect appressoriation. In some way the presence of a hard surface stimulates the formation of appressoria (Emmett and Parbery 1975). Amino acids containing sulfhydryl groups (Netolitzky and Skoropad 1971) or cAMP inhibit appressoriation (Section III). Various other nutritional factors have been shown to variously enhance or inhibit appressoriation (Emmett and Parbery 1975). Any theory that proposes to explain why appressoria form at specific locations must take into account at least the first three pieces of evidence. Also it

is necessary for the conidia of C. graminicola to be in a film of water in order to germinate.

A strictly physical theory which could explain the location of appressoriation is as follows. Due to the hydrophobic nature of natural leaf surfaces, or the nail polish and epoxy replicas, the infection droplet does not wet the surface. Due to the ridged nature of the surface the water droplet probably touches only the top portion of the cell ridges. The grooves over the anticlinal walls are not likely to be wetted i. e. there will be a pocket of air in the groove. Therefore appressoriation could be a response of the germ tube to the antagonistic conditions of the air pocket which it meets when it grows out of the water infection droplet. This theory explains a physical method by which the germ tube can find the anticlinal groove but it does not take into account the effect of a hard surface or various chemicals.

The knowlege that the appressorial adhesive is a hemicellulose can throw some light on the old argument as to whether penetration is mechanical or enzymatic in nature. It would be strongly to the disadvantage of the fungus to secrete a hemicellulase which would degrade its own adhesive. Therefore it would seem that the penetration of hemicellulose must be mechanical. The mechanical advantage is with the fungus since the adhesive area of the fungus is much greater than the penetration peg area which is pushing through the host hemicellulose. It is safe for the fungus to

break the other cuticle and wall materials down enzymatically. This is in agreement with EM work which suggests that both mechanical and enzymatic factors are operating during penetration (Politis and Wheeler 1973).

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APPENDIX

This Appendix contains, in graphical form, all of the data discussed in Section I. They show that the Gompertz equation can serve as a mathematical model of germination and appressoriation in C. graminicola. Both the data points and the regression lines are shown in each graph. The conidia tested were grown under identical conditions except that the parent cultures were exposed to different day lengths as noted at the bottom of each figure.

In general closed symbols denote germination and open symbols denote appressoriation.

FIGS. 1-3. Germination and appressoriation obtained with conidia from cultures of C. graminicola grown under a 6 hour day length.

FIGS. 4-6. Germination and appressoriation obtained with conidia from cultures of C. graminicola grown under a 12 hour day length.

FIGS. 7-9. Germination and appressoriation obtained with conidia from cultures of C. graminicola grown under an 18 hour day length.

FIGS. 10-12. Germination and appressoriation obtained with conidia from cultures of C. graminicola grown under a 24 hour day length.

KEY

Germination:

1=△ , 2=◇ , 3=⊙ , 4=⊠ , 5=⧖ , coincident points.

Appressoriation:

1=+ , 2=X , 3=Y , 4=Z , 5=* , coincident points.

In all cases the upper line is that found by regression to the Gompertz equation for germination. Similarly the lower line is that for appressoriation.

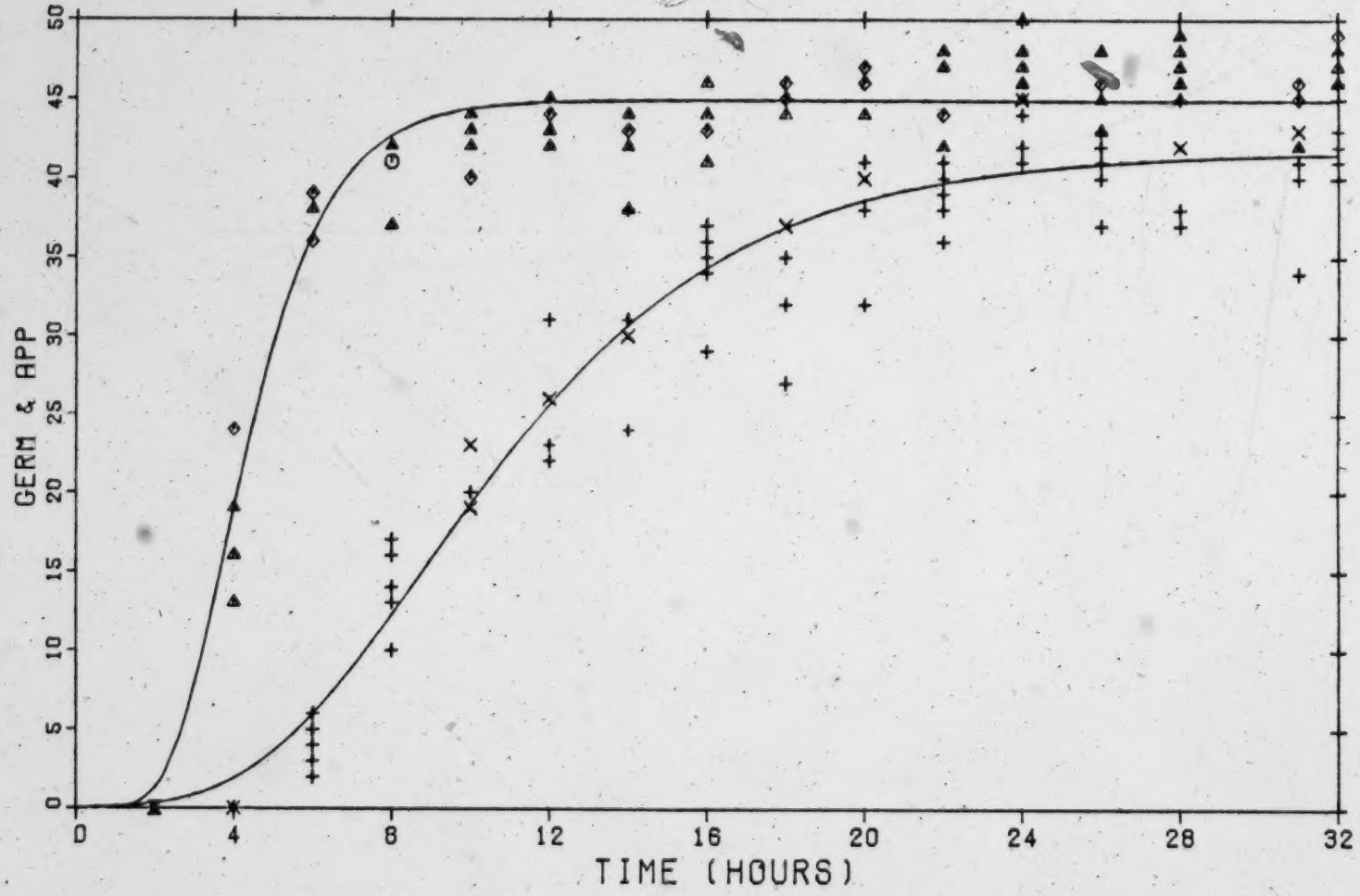

FIG. 1 PARENT CULTURE 6 HOURS OF LIGHT

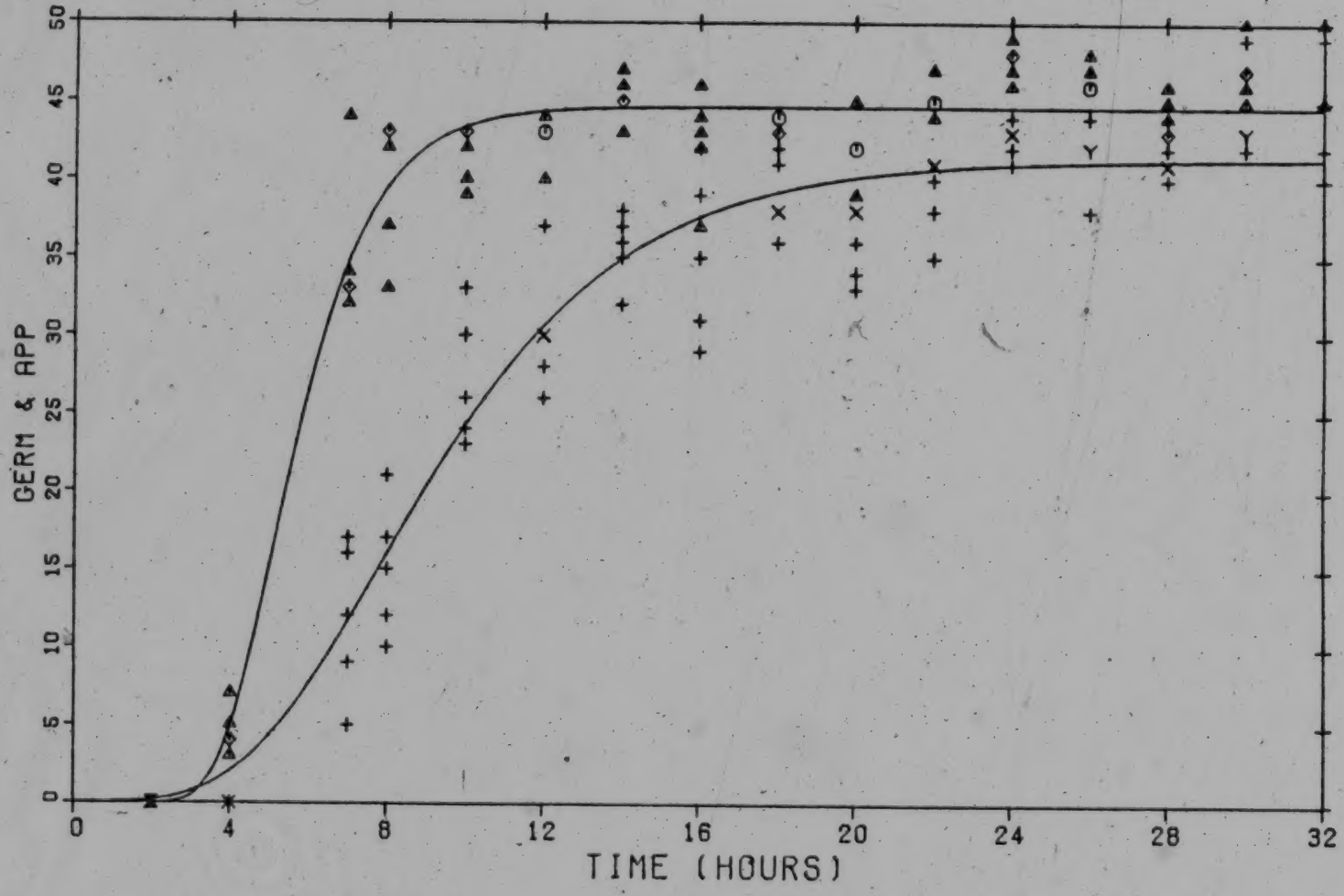

FIG. 2 PARENT CULTURE 6 HOURS OF LIGHT

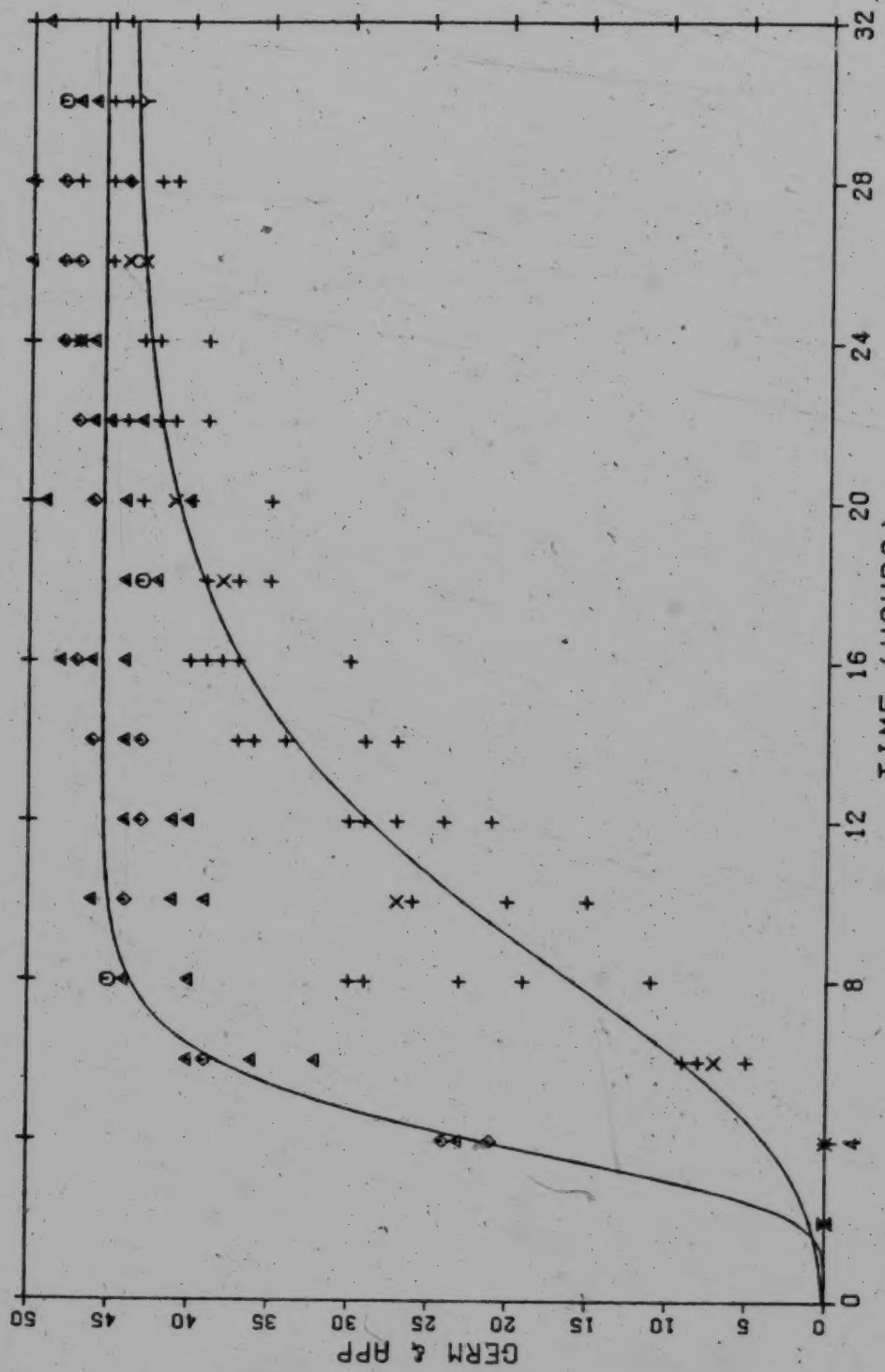

FIG. 3 PARENT CULTURE 6 HOURS OF LIGHT

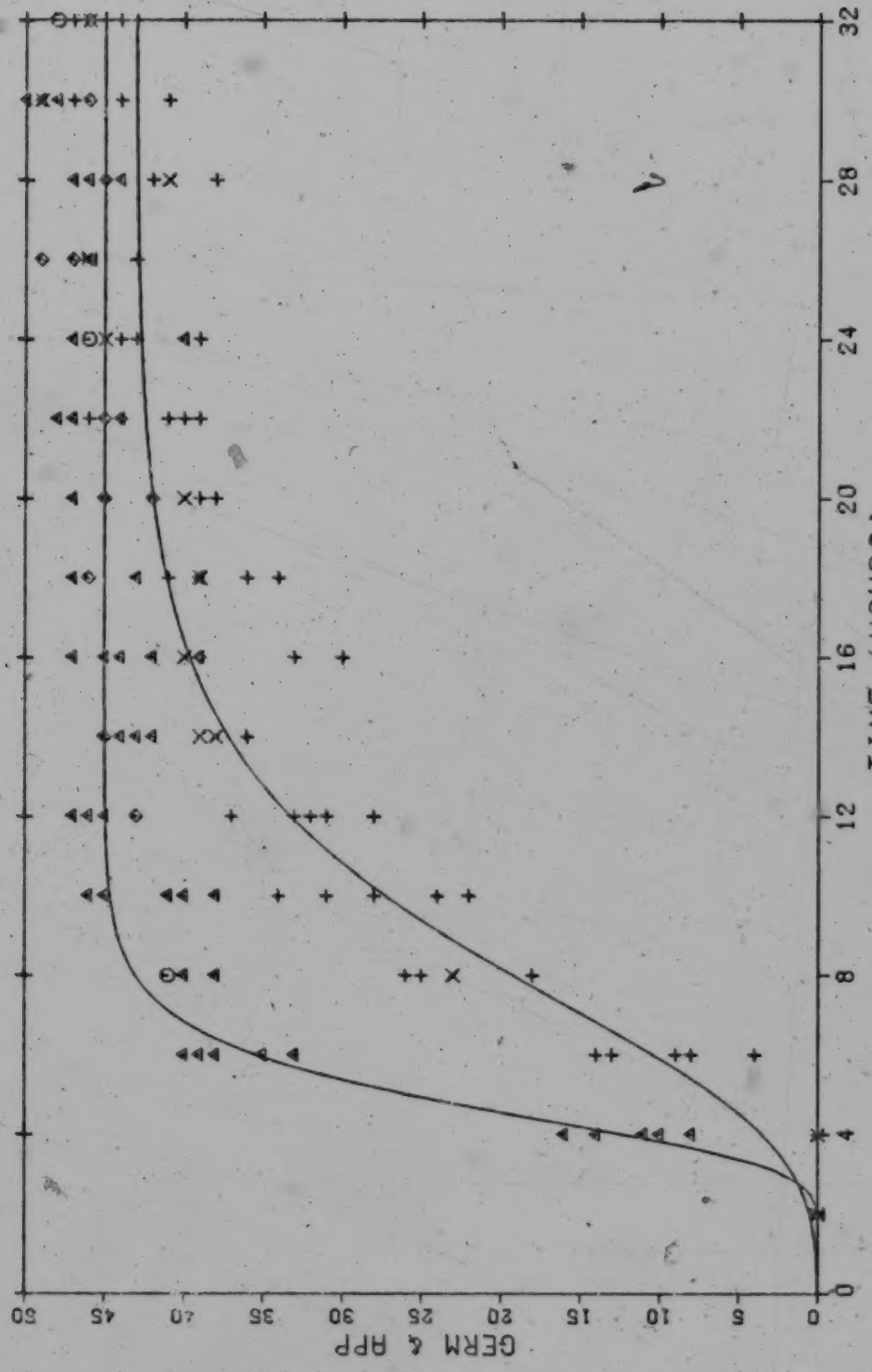

FIG. 4 PARENT CULTURE 12 HOURS OF LIGHT

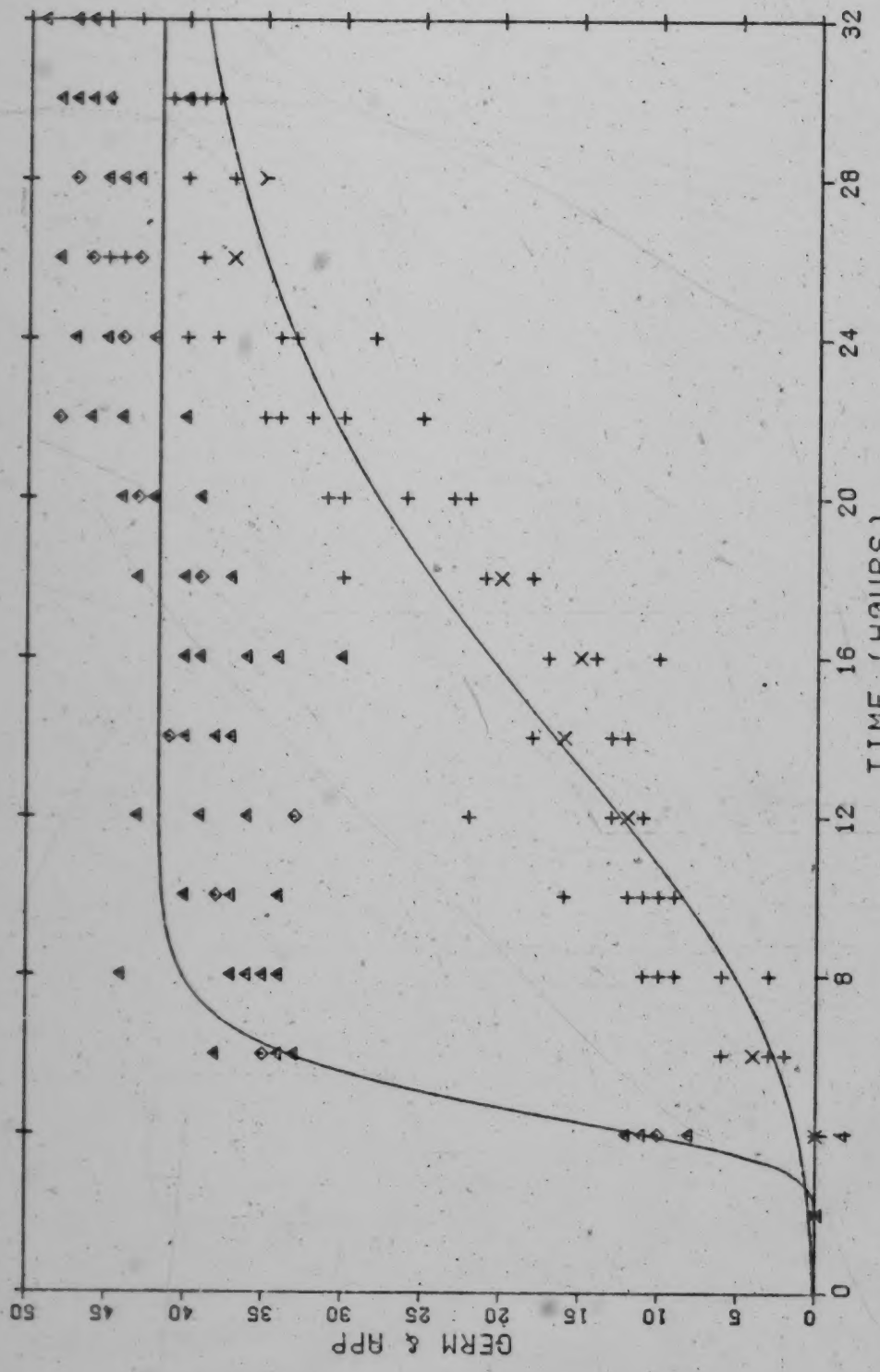

FIG. 5 PARENT CULTURE 12 HOURS OF LIGHT

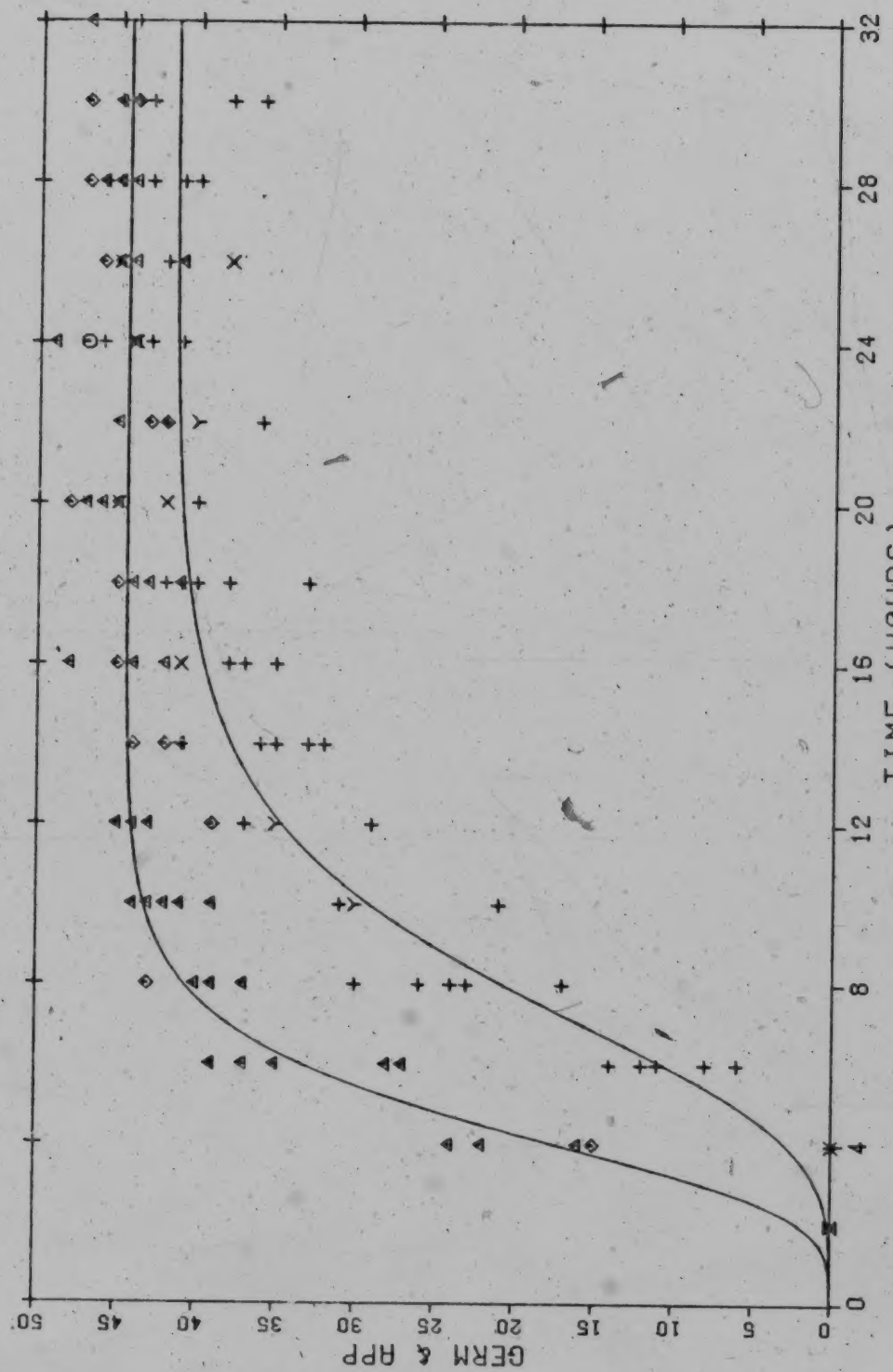

FIG. 8 PARENT CULTURE 12 HOURS OF LIGHT

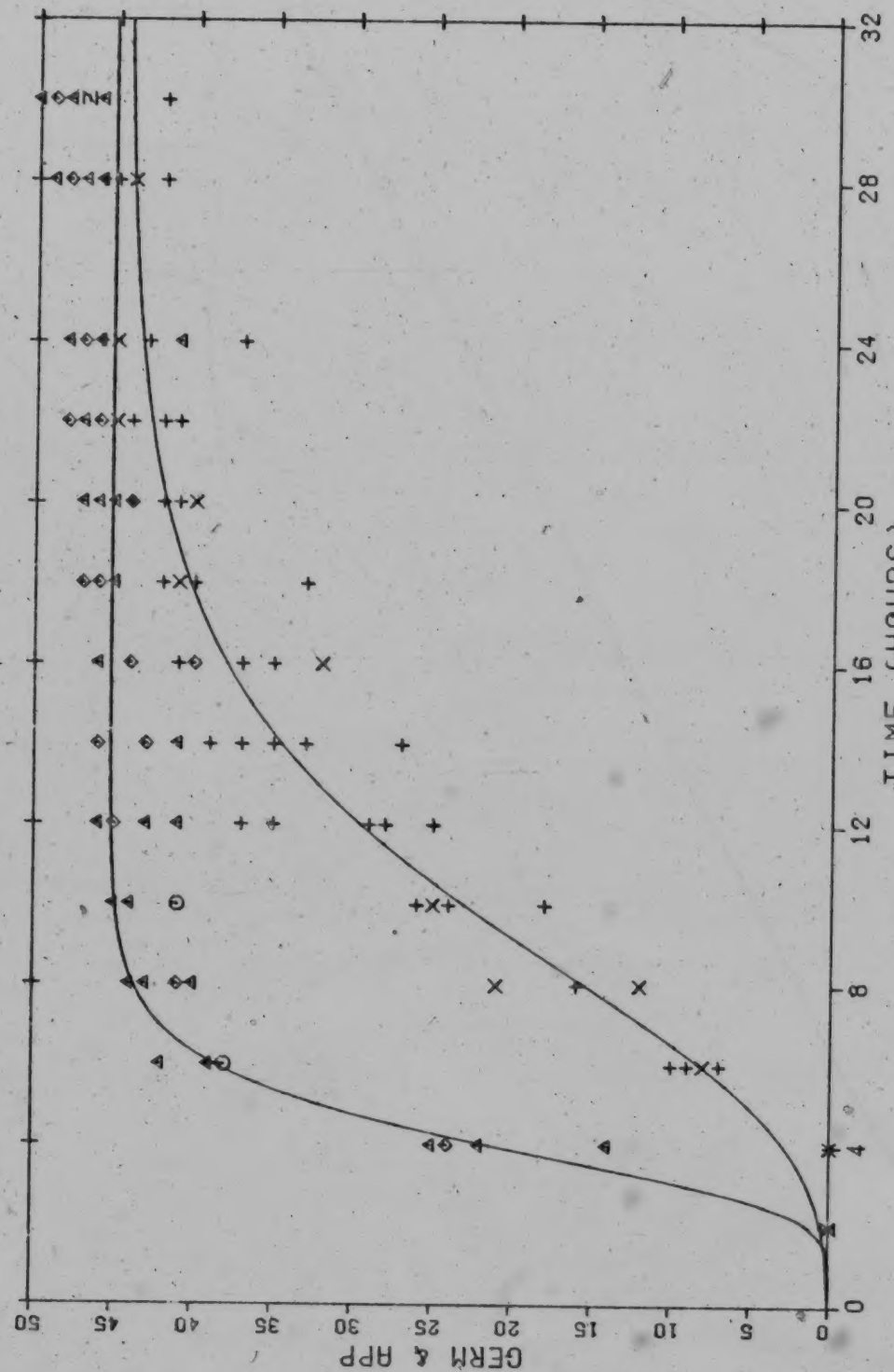

FIG. 7 PARENT CULTURE 18 HOURS OF LIGHT

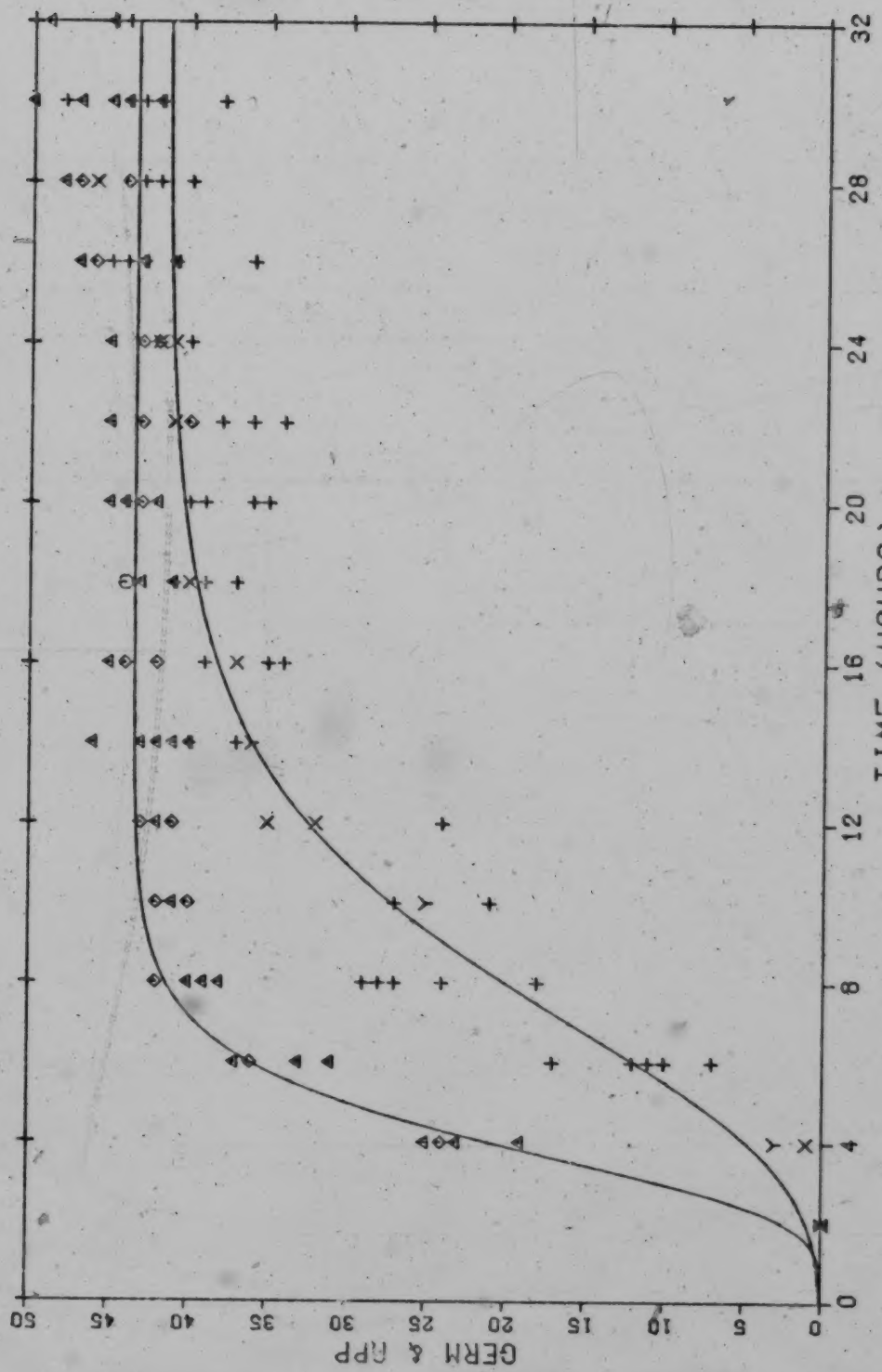

FIG. 8 PARENT CULTURE 18 HOURS OF LIGHT

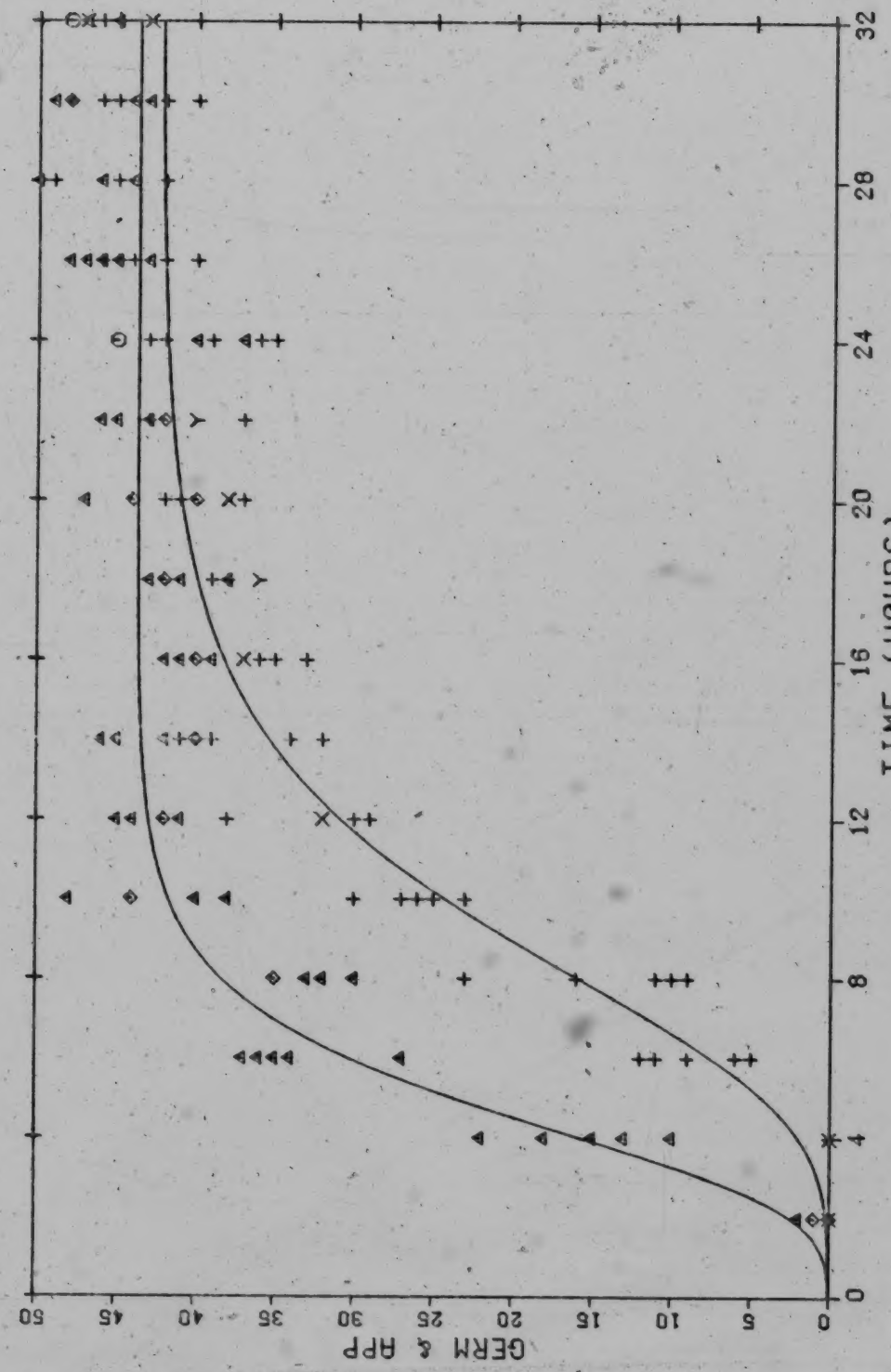

FIG. 9 PARENT CULTURE 18 HOURS OF LIGHT

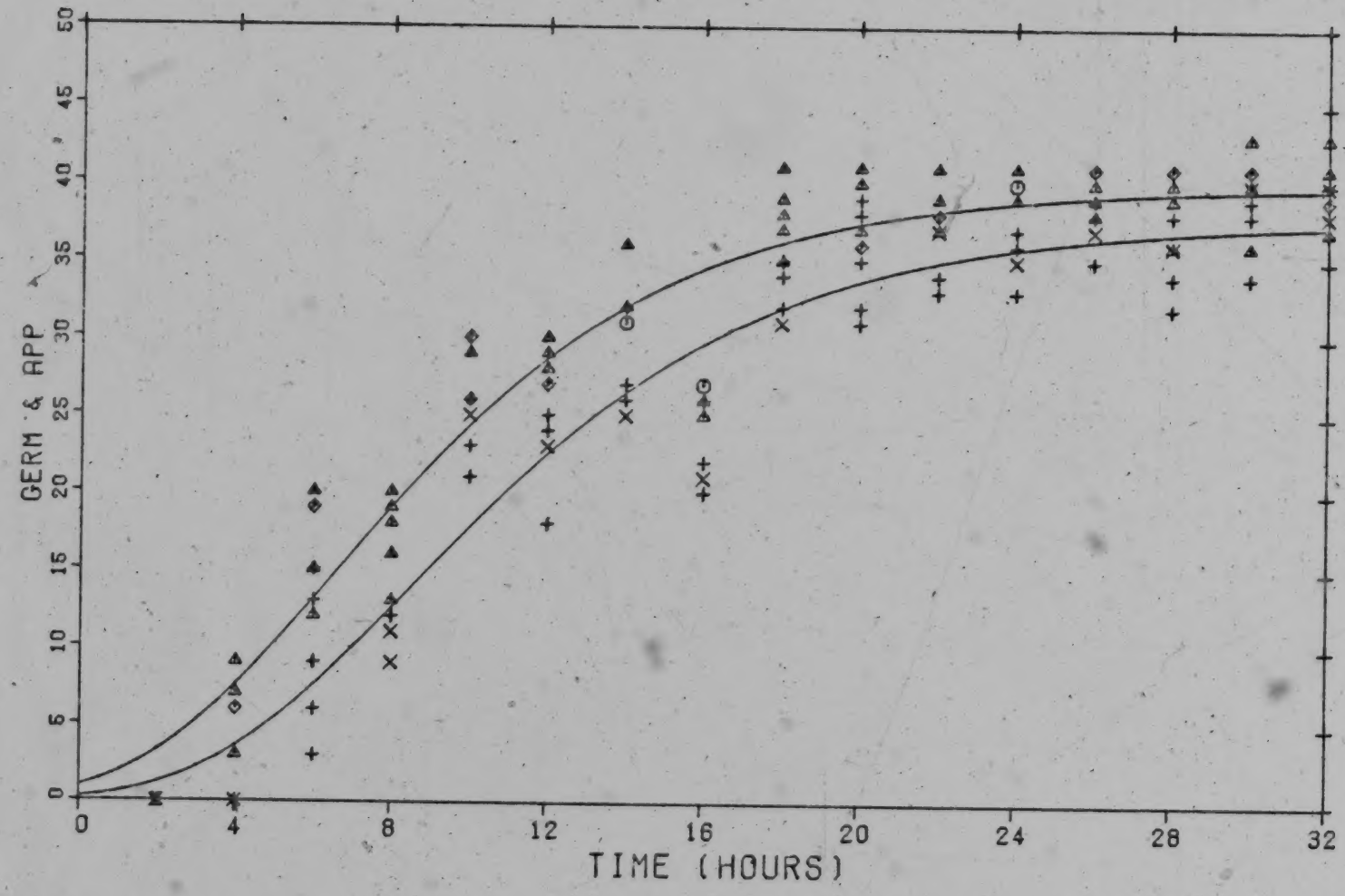

FIG. 10 PARENT CULTURE 24 HOURS OF LIGHT

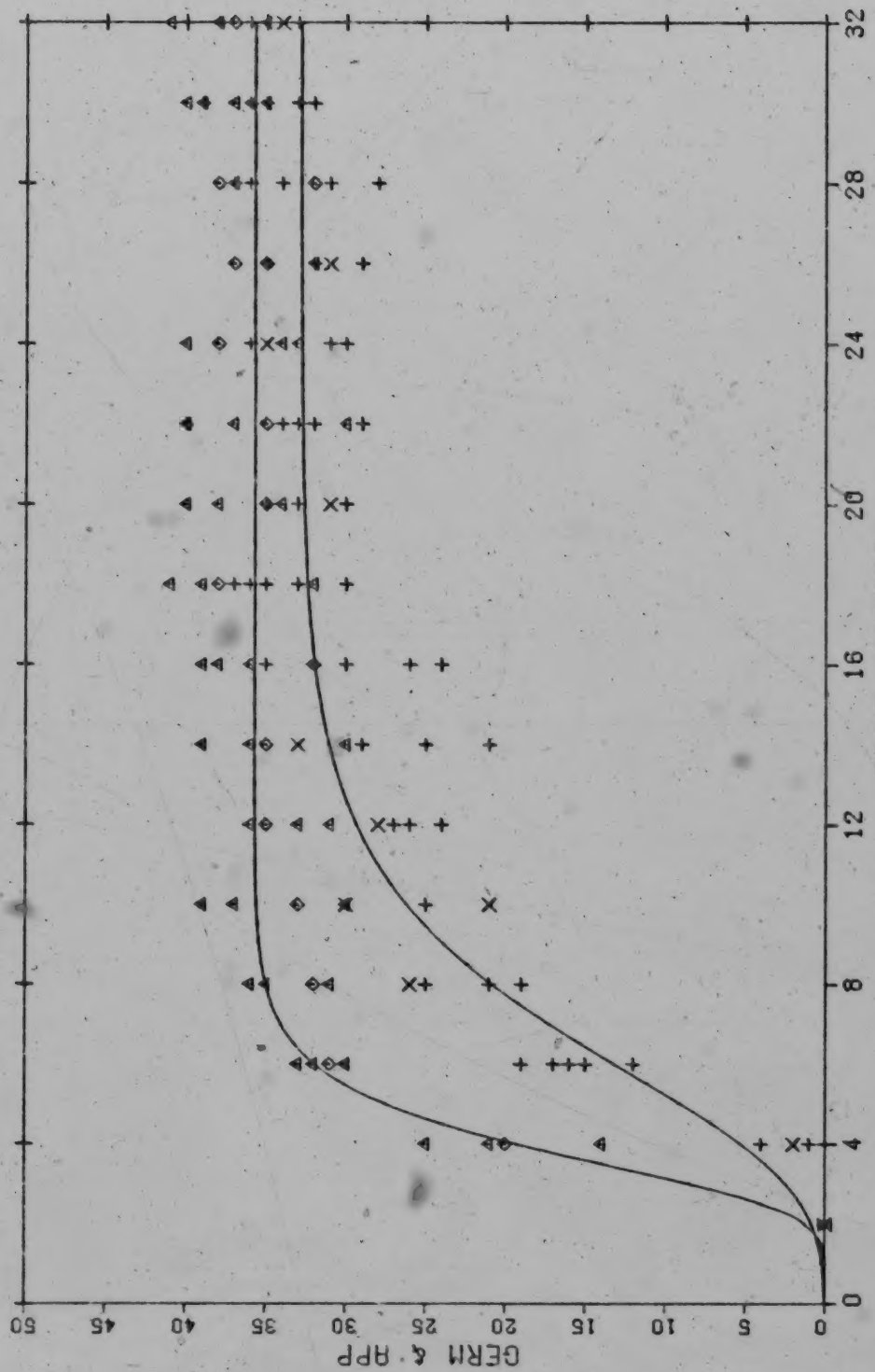

FIG. 11 PARENT CULTURE 24 HOURS OF LIGHT

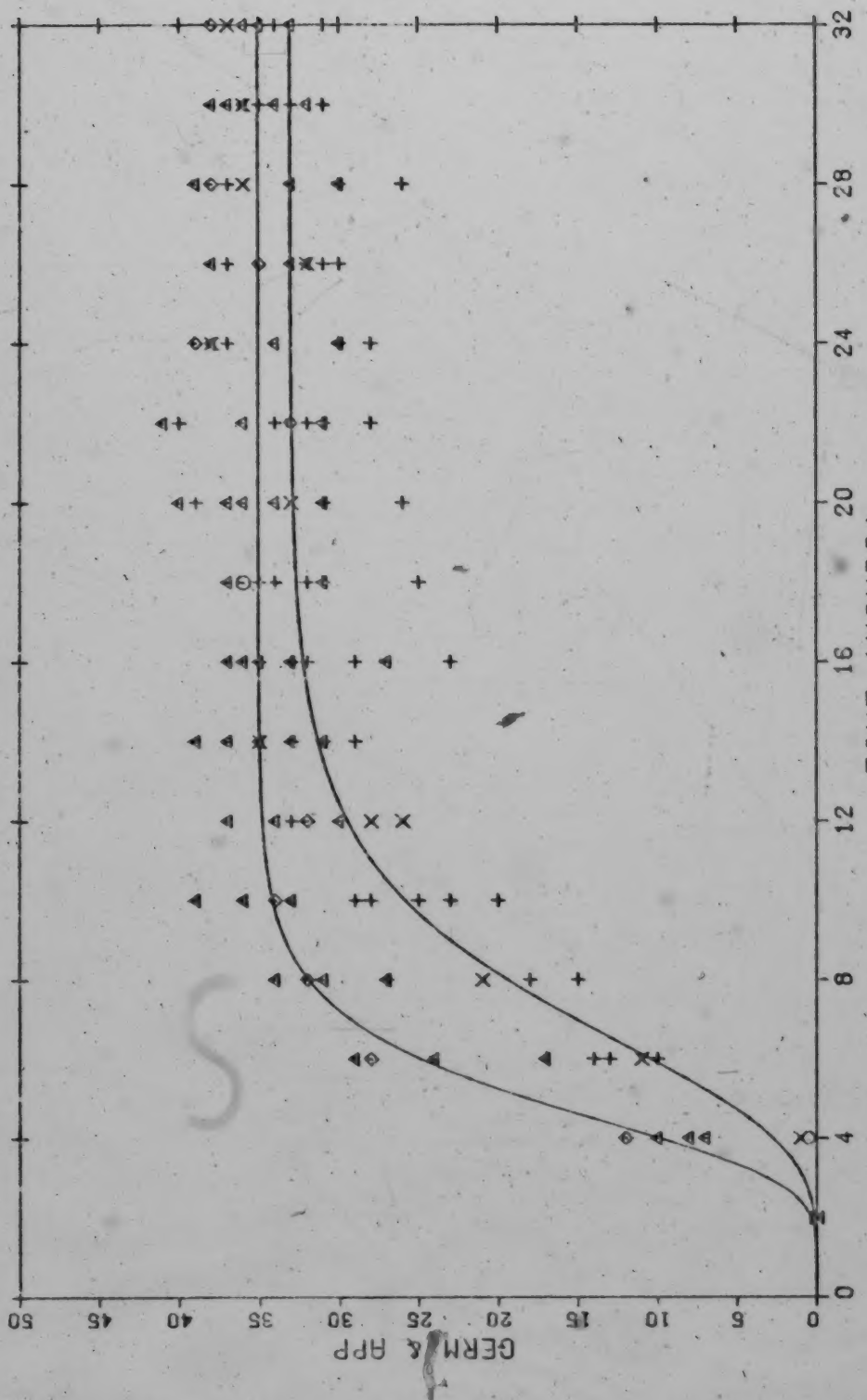

FIG. 12 PARENT CULTURE 24 HOURS OF LIGHT